Standard Operating Procedures for the Hach DR 5000 Spectrophotometer

I. DR 5000 Spectrophotometer

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1. Task:

- All water sources in the field should be considered unsafe until evaluated by preventive medicine personnel and approved by the command medical authority. The Inficon HAPSITE GC/MS and Hach Dr 5000 UV-Vis Spectrophotometer give the FDPMU water quality analysis (WQA) capability that far exceeds that of any Service's organic preventive medicine units. The FDPMU provides an intermediate level of testing between basic potability testing conducted by organic preventive medicine personnel and advanced water testing performed by rear

echelon laboratories. This enhanced, in-theater capability provides operation commanders and the command medical authority far greater confidence in the safety of field drinking water supplies both through initial assessment and continued surveillance. Target analytes for FDPMU WQA based on the Tri-Service Field Water Standards and U.S. Environmental Protection Agency National Primary Drinking Water Regulations are listed in table 1 below.

Note: This Standard Operating Procedure provides basic set-up and operating procedures as well as QA and sample preservation requirements for water quality analyses performed on the DR 5000 Spectrophotometer. The Hach DR 5000 User Manual and Methods contain more detailed information and additional operational procedures for optimizing instrument performance.

Table 1- Target Analytes for FDPMU Water Quality Analysis

Alachlor	Atrazine	Arsenic	Barium
Benzene	Cadmium	Carbon tetrachloride	Chloride
Chlorobenzene	Chromium (total)	Copper	Cyanide
o-Dichlorobenzene	p-Dichlorobenzene	1,2 Dichloroethane	1,1 Dichloroethylene
cis-1,2 Dichlorobenzene	trans-1,2 Dichlorobenzene	Dichloromethane	1,2 Dichlorpropanne
Ethylbenzene	Ethylene dibromide	Fluoride	Lead
Magnesium	Mercury	Nitrate	Nitrite
Selenium	Styrene	Sulfate	Tetrachloroethylene
Toluene	1,2,4 Trichlorobenzene	1,1,1 Trichlorethane	1,1,2 Trichloroethane
Trichloroethylene	Trihalomethanes (total)*	Total/Fecal Coliforms	Turbidity
Vinyl chloride	Xylenes (total)	Total Dissolved Solids	Color

Table 2 lists those analytes performed on the Hach DR 5000 UV-Vis Spectrophotometer.

Table 2 - DR 5000 Spectrophotometer UV-Vis Target Analytes

Alachlor	Atrazine	Arsenic	Barium
Cadmium	Chloride	Chromium (total)	Color
Copper	Cyanide	Fluoride	Lead
Magnesium	Mercury	Nitrate	Nitrite
Selenium	Sulfate	Trihalomethanes (total)	

*Individual trihalomethane compounds can be identified and quantified via the Inficon HAPSITE as required.

2. Principles of Operations:

- The DR 5000 Spectrophotometer is a scanning UV/VIS spectrophotometer with a wavelength range of 190 to 1100 nm. The instrument comes with a complete set of application programs and multi-language support.
    - The DR 5000 is used for testing in visible and ultraviolet wavelengths. A gas-filled tungsten lamp produces light in the visible spectrum (320 to 1100 nm), and a deuterium lamp produces light in the ultraviolet spectrum (190 to 360 nm).
    - The DR 5000 Spectrophotometer provides digital readouts in direct concentration units, absorbance, or percent transmittance. When a user-generated or programmed method is selected, the on-screen menus and prompts direct the user through the test.
    - This menu system can also be used to generate reports, statistical evaluations of generated calibration curves, and to report instrument diagnostic checks.
    - Running an analysis with the DR 5000 is relatively simple and involves

        1) Zeroing the instrument by measuring the amount of light passing through a sample with no reagents added
        2) Running a Reagent Blank which is a sample cell filled with de-ionized water to which reagents are added
        3) Reading the sample where the instrument measures the amount of light passing through a reacted sample and converts the transmitted light into a concentration

Note: Reagent Blanks are only required only when opening new reagents.

3. Operating Environment

CAUTION: The lamp cover can become hot, especially when the deuterium lamp is used. Do not place anything on top of the cover.

Important Note: Protect the instrument from temperature extremes, including heaters, direct sunlight, and other heat sources.

- The following conditions are necessary to ensure that the instrument runs accurately and has a long life span.
    - Place the instrument firmly on an even table surface. Do not push any objects
    - under the instrument, they can block the ventilation slits.
    - Maintain an ambient temperature of 10 to 40 °C (50 to 104 °F) for proper
    - instrument operation.
    - The relative humidity should be less than 80%; moisture should not condense on the instrument.
    - Leave at least a 15 cm (6 inch) clearance at the top and on all sides for air circulation to avoid overheating of the electrical parts.

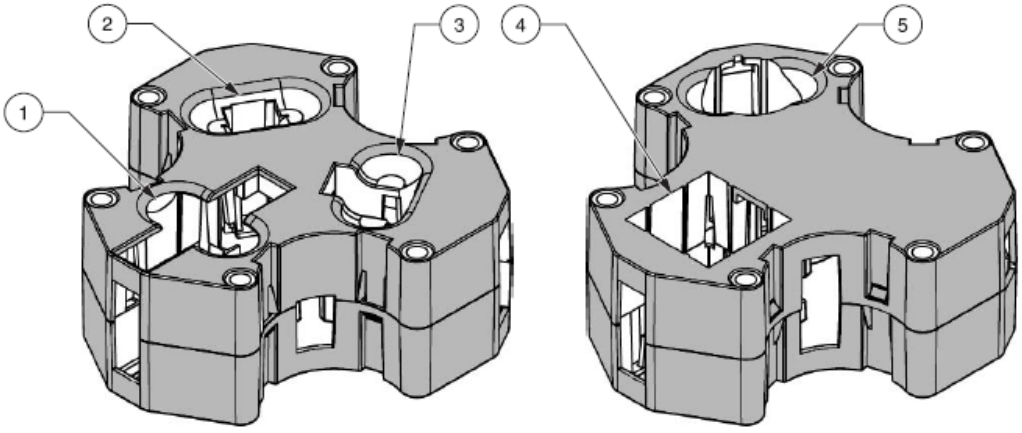
4. Instrument Start-up

Installing the Multi-Cell Adapter

- The DR 5000 Spectrophotometer comes equipped with a Multi-Cell Adapter (Figure X) which is the standard holder supplied with each instrument. On the top and bottom of the Multi-Cell Adapter are a variety of openings to accommodate different types of cells. Each opening is labeled for the type of cell. The Multi-Cell Adapter can accommodate the following cell types:

    - 10/20/50 mm rectangular cells
    - 1-inch round cells
    - 1-inch square cells

- Only one cell type can be used for a measurement at one time.



1. 50 mm Rectangular Cell	**3.** 20 mm Rectangular Cell	**5.** 1-inch Round Cell
2. 10 mm Rectangular Cell	**4.** 1-inch Square Cell	

Figure 1 - Multi-Cell Adapter

- Installation -

    - Open the cell compartment.
    - Identify the correct opening for the selected cell type in the Multi Cell Adapter.
    - Insert the Multi-Cell Adapter in the cell compartment in such a way that the name of the selected cell type can be read directly and the cell opening is at the front.
    - Locate the adapter on the two conical pins, and secure with the two locking screws (Figure 2).

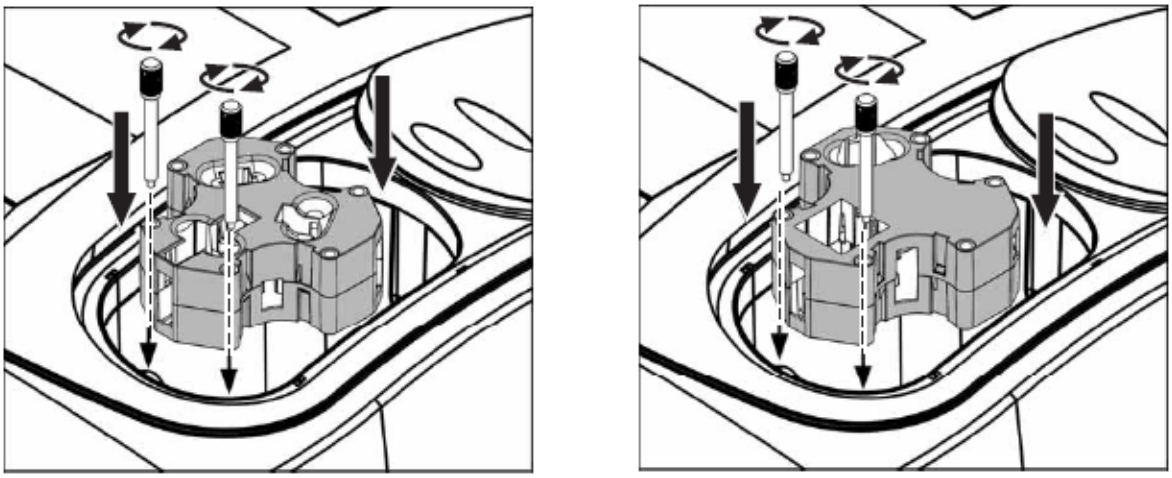


Figure 2 - Multi-Cell Adapter Installation

- Turning on the Instrument

  - Plug in the power supply cord.
  - Close the empty cell compartment.
  - Press the On/Off switch on the back of the instrument to power the instrument.

  Note: Do not switch the instrument off and on in rapid succession. Always wait at least 20 seconds before switching the instrument on again; otherwise damage to the electronic and mechanical systems may occur.

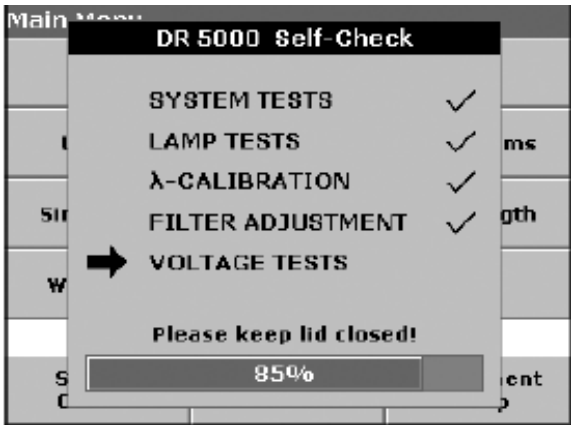
- Language Selection - The DR 5000 Spectrophotometer software includes several language options. The first time the instrument is powered on, the language selection screen will appear.

  - Select the desired language.

  - Press **OK** to confirm the language selection. The self check will start automatically.

- System Diagnostics - Each time the instrument is powered up, a series of diagnostic tests are performed automatically to ensure operation of major system components.

This procedure, which takes approximately two minutes, checks the system, lamps, wavelength calibration, filter adjustment, and voltage. Each component that functions correctly is confirmed with a check mark. The Main Menu is displayed when the system diagnostics are complete. Refer to Section 8 on page 105 of the User Manual for troubleshooting information if any error messages are displayed during the system diagnostics.



5. Basic Operation[+]:

- Getting Started

  - Tips for Using the Touch Screen

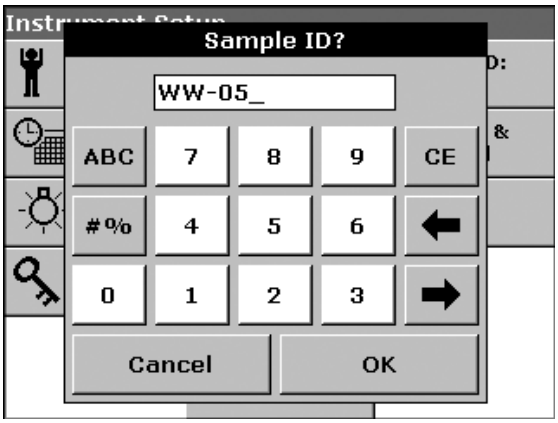
    1) The entire screen is touch-activated. To make a selection, press the screen with a fingernail, fingertip, pencil eraser, or a stylus.
    2) Do not press the screen with a sharp object, such as the tip of a ball point pen.
    3) Do not place anything on top of the screen, to prevent damage or scratching on the screen.
    4) Press keys, words, or icons to select them.
    5) Use scroll bars to move up and down long lists very quickly. Press and hold the scroll bar, then move up or down to move through the list.
    6) Highlight an item from a list by pressing it once. When the item has been successfully selected, it will be displayed as reversed text (light text on a dark background).

[+] ***For other and/or more advanced operations consult the DR 5000 User's Manual***

- Using the Alphanumeric Keypad -

  - This display is used to enter letters, numbers, and symbols as needed when programming the instrument. Unavailable options are disabled (grayed out). The icons on the right and left of the screen are described in Table 3.

- The central keypad changes to reflect the chosen entry mode. Continue to press a key until the desired character appears on the screen. A space can be entered by using the underscore on the **YZ_** key.



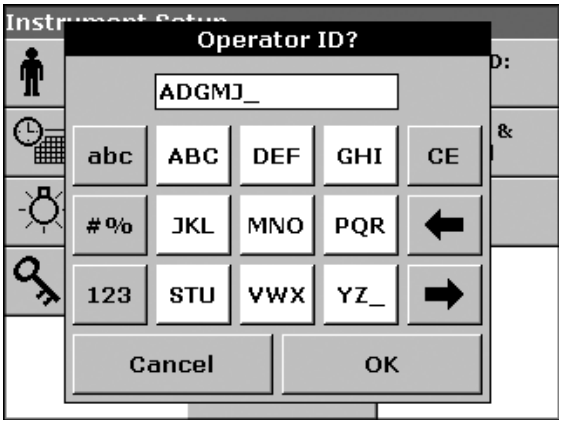


Table 3 - Alphanumeric Keypad Functions

Icon	Description	Function
ABC	Alphabetic	When entering alphabetic characters (e.g. user-entered units), toggles between upper and lower case letters.
#%	Symbols	Punctuation, symbols, and numerical sub- and superscripts may be entered.
123	Numeric	Enters regular numbers.
CE	Clear Entry	Clears the entry.
LEFT ARROW	Backspace	Moves back one position. Deletes the character previously entered in the new position.
RIGHT ARROW	Advance	Moves to the next space in an entry when two adjacent characters occur on the same key.

- DR 5000 Main Menu and Display -

  - All current selection and input options, analysis results, and scans are shown in the graphic display. The display changes as different modes of operation are selected.

  - The Main Menu (Figure 3) appears when the instrument is powered on. A variety of options may be selected from the Main Menu. Table 4 on the following page briefly describes each menu option.

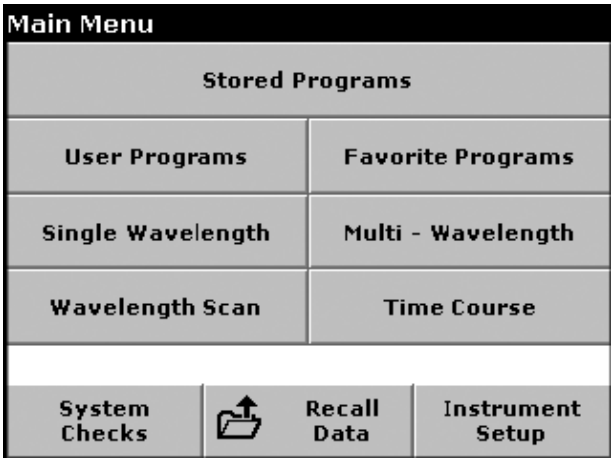


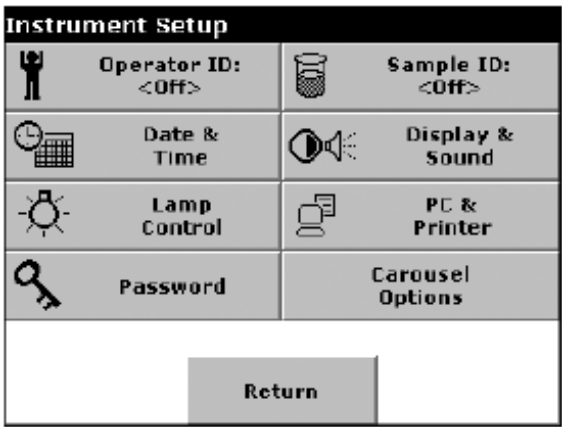
Figure 3 – Main Menu

Table 4 - Main Menu Options	
Soft Key	**Function**
STORED PROGRAMS	Stored programs are pre-programmed methods that make use of reagents, cuvette tests, and pipette tests. Step-by-step procedures for analyses of FDPMU target analytes using stored programs are supplied below.
USER PROGRAMS	User programs make "made to measure analysis" possible: Users can program custom methods. Stored methods can be saved as user programs. The tests can then be modified to suit the user's requirements.
FAVORITE PROGRAMS	List of frequently-used methods, placed in this area for easy access.
SINGLE WAVELENGTH	Single wavelength measurements are: **Absorbance Measurements:** The light absorbed by the sample is measured in absorbance units. **Transmittance Measurements (%):** The percentage of the light that passes through the sample and reaches the detector is measured. **Concentration Measurements:** A concentration factor can be entered to enable the measured absorbance values to be converted into concentration values.
MULTI-WAVELENGTH	Absorbance (Abs) or percentage transmittance (%T) is measured at up to four wavelengths, and absorbance differences and absorbance relationships are calculated. Simple conversions into concentrations can also be performed.
WAVELENGTH SCAN	A wavelength scan shows how the light from a sample is absorbed over a defined wavelength spectrum. This function can be used to determine the wavelength at which the maximum absorbance value can be measured. The absorbance behavior is displayed graphically during the scan.

Table 4 - Main Menu Options, continued	
TIME COURSE	The time scan records the absorbance or % transmittance at a wavelength over a defined time.
SYSTEM CHECKS	The system checks menu offers a number of options, including optical checks, output checks, lamp history, and instrument update.
RECALL DATA	Stored data can be recalled, filtered, transmitted, and deleted.
INSTRUMENT SETUP	User-specific or method-specific settings can be entered: Operator-ID, Sample-ID, Date & Time, Display & Sound, Lamp Control, Password, and PC & Printer.

- Instrument Setup Mode

  - The Instrument Setup menu can be viewed from the Main Menu by selecting **INSTRUMENT SETUP**. From the reading mode, select Options.
  - A number of functions/options are displayed, which can be used to enter basic instrument settings. This display appears when the multi-cell holder is installed or the 13 mm/16 mm round vial compartment is used. Additional keys are displayed if optional modules are installed (i.e. carousel options).

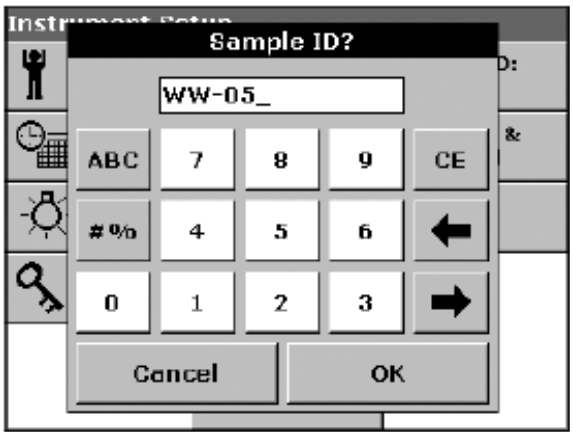


- Setting the Operator ID - Use this option to enter up to 30 sets of operator initials (up to five characters each) into the instrument. This feature helps record which operator measured each sample.
  - From Instrument Setup, select **OPERATOR ID**.
  - Press **NEW** to enter a new Operator ID.
  - Use the alphanumeric keypad to enter a new Operator ID. Press **OK** to confirm. Spaces are not available in this function. Use the underscore symbol instead.
  - The display shows the chosen Operator ID. Press **OK**.

- Alternatively, if an Operator ID is active, press the **OPERATOR ID** icon on the Measurement screen. The Operator ID screen will appear and allow changes to the ID.

- Modifying an Operator ID - Press the **OPERATOR ID** icon on the Measurement screen. The Operator ID screen will appear and allow changes to the ID.
  - Deleting an Operator ID - To delete an operator, select the Operator ID and press **DELETE.**
- Setting the Sample ID - Use this option to enter up to 30 sample identifications (up to 13 characters each) into the instrument. Sample IDs are used to specify the sample.

  - From Instrument Setup, select **SAMPLE ID**.
  - Press **NEW** to enter a new Sample ID.
  - Use the alphanumeric keypad to enter a new Sample ID. Press **OK** to confirm.



  - To number the Sample IDs sequentially (e.g. Inflow 01...etc.), select **ADD NUMBER**.
    1) Use the arrow keys to specify the first number in the sequence.
    2) (b) Use the key between the arrow keys to enter the first number of the sequence using the alphanumeric keypad.

    3) Press **OK** to confirm and return to the Instrument Setup. The Sample ID is activated. Each Sample ID is automatically numbered in ascending order

after a measurement. The number is shown in parentheses behind the Sample ID.

4) Alternatively, if a Sample ID is active, press the **SAMPLE ID** icon on the measurement screen. The Sample ID screen will appear to allow changes to the Sample ID.

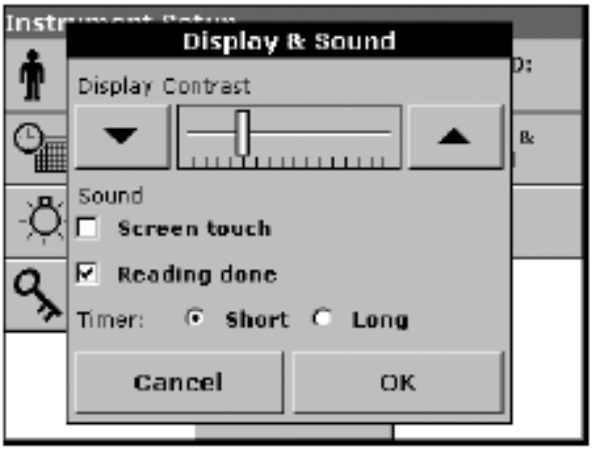
- Setting the Date and Time

    - From Instrument Setup, select **DATE & TIME**. The date and time are subdivided over a number of fields.

(2) Select the appropriate field and use the arrow keys to change the value. Press **OK** to confirm and return to Instrument Setup.

- Setting the Display and Sound Preferences - From Instrument Setup, press **DISPLAY & SOUND**. The following options will appear:

    - Display/Contrast—Adjusts the display contrast to suit lighting conditions and viewing angle.
    - Screen Touch—Activates a short beep each time the screen is pressed (Default: off).
    - Reading Done—Activates/Deactivates a sound when a reading is complete (Default: short beep every time a reading is complete).
    - Timer—Adjusts the length of the timer sound. Select Short or Long. Long beeps are recommended for noisy environments.

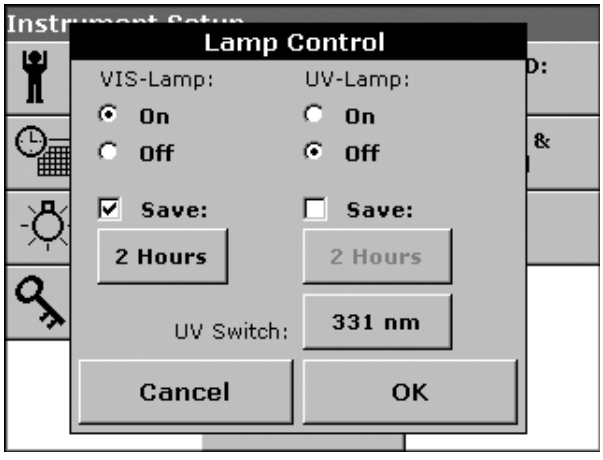


- Press **OK** to confirm and return to Instrument Setup.

- Setting the Lamp Control

  - The tungsten lamp produces light in the visible spectrum (320 to 1100 nm).
  - The deuterium lamp (UV-lamp) produces light in the ultraviolet spectrum (190 to 360 nm).
  - In the overlap zone from 320 to 360 nm, either the deuterium lamp (UV-lamp) or the tungsten lamp can be used for measurements.
  - The performance of the lamps is impacted by on-off operation and the length of use. For maximum performance, turn the lamp off only if it will remain off for at least 4-5 hours.
  - The lamp switches on automatically if a lamp is needed for the selected program or if the instrument is operating inside the lamp spectrum.

    1) From Instrument Setup, press **LAMP CONTROL**.
    2) Select **On** to toggle on the VIS-Lamp or UV-Lamp, respectively.
    3) Activate Save to define the length of time for which the VIS-Lamp or the UV- Lamp will be powered on.

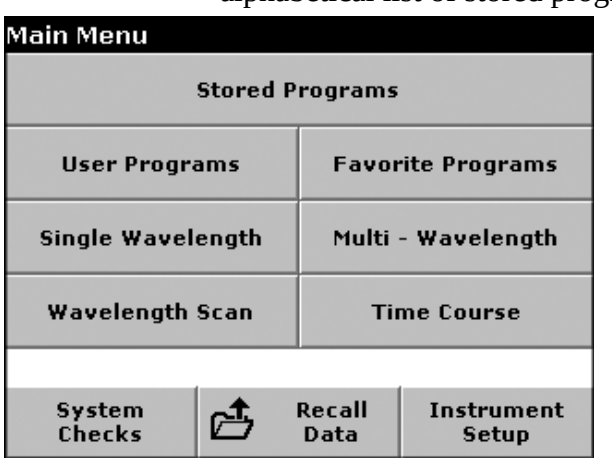


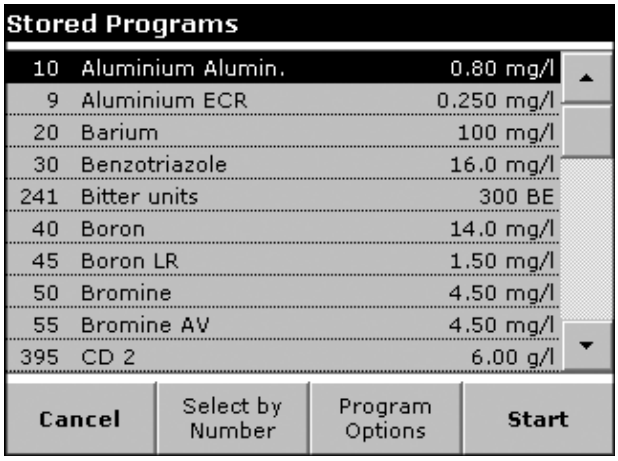
4) Select the length of time the lamp will be switched on. After this period of time the lamp will automatically power off after no measurements are made. Press **OK** to confirm.
5) Press the **UV SWITCH** key to select the wavelength value between 320 nm and 360 nm, at which the instrument changes from the visible to UV source.
6) Use the alphanumeric keypad to enter the maximum wavelength for UV operation. Press **OK** to confirm and return to Lamp Control.
7) Press **OK** to confirm and return to Instrument Setup.

Note: If a lamp is turned off in Lamp Control, it will automatically power on if it is needed for a stored program. If the UV lamp is powered off it will take approximately 3 minutes to warm up. During this time the instrument will display "Lamp Warm-up."

- Stored Programs - The DR 5000 Spectrophotometer contains programmed procedures that can be accessed through the Stored Programs menu.

  - Selecting a Stored Program
    1) From the Main Menu, press STORED PROGRAMS to view an alphabetical list of stored programs.

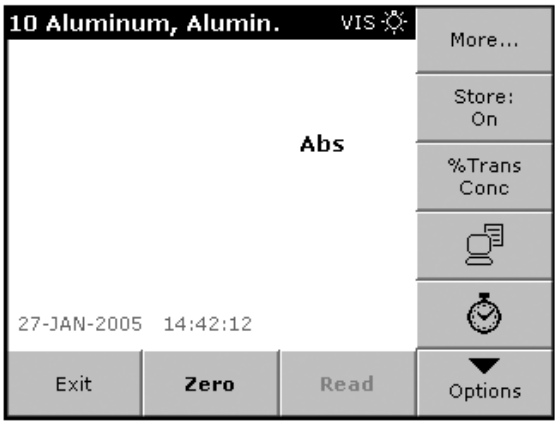




    2) Select the program number by name or use the arrow keys to scroll through the list quickly and highlight the program or press **SELECT BY NUMBER** to search for a number. Use the alphanumeric keypad to enter the test number and press **OK** to confirm.
    3) Press **START** to run the program. After a program is selected, the screen for that analyte will appear. The wavelength does not need to be selected. Only the options appropriate to the method will be displayed in black. Unused options will be grayed out or will not appear.

  - Stored Program Options
    1) From the Main Menu, select **STORED PROGRAMS.** Choose the desired method and press **START**.

2) Press **OPTIONS** to access data storage, readings, concentration, or wavelength setup options. Press **MORE** to view additional setup options. Refer to Table 5 below for descriptions.



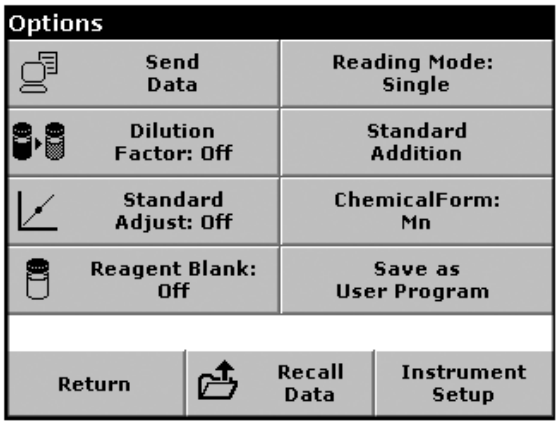


Table 5 - Stored Program Options

Option	**Description**
Store Off/On	With **STORE ON** selected, all measurement data are stored automatically. With **STORE OFF** selected, no measurement data are stored.
% Trans/Abs/Conc	Toggle between % transmittance, absorbance readings, or concentration.
	Absorbance: Measures the amount of light absorbed by the sample, in units of Absorbance.
	% Transmittance: Measures the percent of the original light that passes through the sample and reaches the detector.
	Concentration: Displays the results in concentration.
Send Data	Icon Sends data to a printer, computer, or USB memory stick.
Timer icon	Functions as a stopwatch. Displays pre-set periods for reactions, heating, etc., along with a description of the activity, when appropriate. When the specified time has elapsed, an audible signal is emitted. The timer has no influence on the measurement program.
Reading Mode	Single: A reading is only displayed after a measurement has been performed. The **ZERO** or **READ** key must be pressed to initiate a measurement.
	Continuous: After zero measurement, all readings are displayed automatically and continuously (default setting). The **READ** key does not appear.

Table 5 - Stored Program Options, continued	
Dilution Factor On/Off	A corrective dilution factor can be entered in order to take into account certain properties. The number entered at the dilution factor prompt will be multiplied by the result to compensate for the adjustment. For example, if the sample has been diluted by a factor of 2, enter 2. The default setting of the dilution factor is 1, corresponding to no dilution. When a dilution is in effect, the **DILUTION** icon will appear on the display.
Standard Addition	Enables the accuracy of the measurements to be checked. The procedure for a test parameter contains a detailed explanation of how to use this function.
Chemical Form	For some stored programs, the chemical form and measuring range can be selected.
Reagent Blank	Enables the reagent blank value to be added to, or subtracted from, the subsequent readings. The reagent blank value shifts the calibration curve along the y-axis (concentration), without changing the shape or gradient of the curve. The effect corresponds to a y-axis intercept of the calibration straight line. This is represented by the equation: Concentration = [(Conc. factor) * Abs] – (reagent blank value).
Save as User Program	Stores the selected parameters as a User Program.
Recall Data	Recalls saved measurement data, wavelength scans, or time courses refer to user's manual for more information).
Instrument Setup	Basic operation settings of the instrument

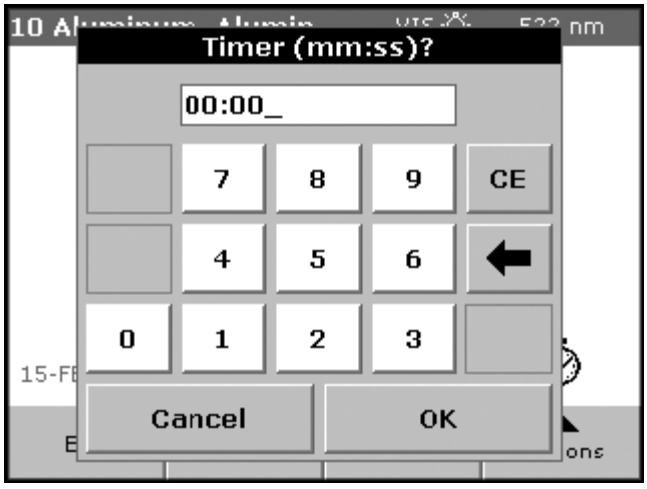
- Using Program Timers - Some procedures do not require the use of timers. Other procedures require several timers. These timers are pre-programmed into each Stored Program, along with a description of the activity to be performed during the timed period.

- Press the **TIMER** (clock) icon on the display. Press **OK** to start the timer. The timer will count down on the screen.

Note: To view the Program screen while the timer is running, press CLOSE. The time will be shown on the bottom left side instead of the date.

- To start the next timed activity for the Stored Program, press the **TIMER** icon and **OK**. If necessary, press **CANCEL** to stop the countdown. The Timer will beep when the timed interval ends.

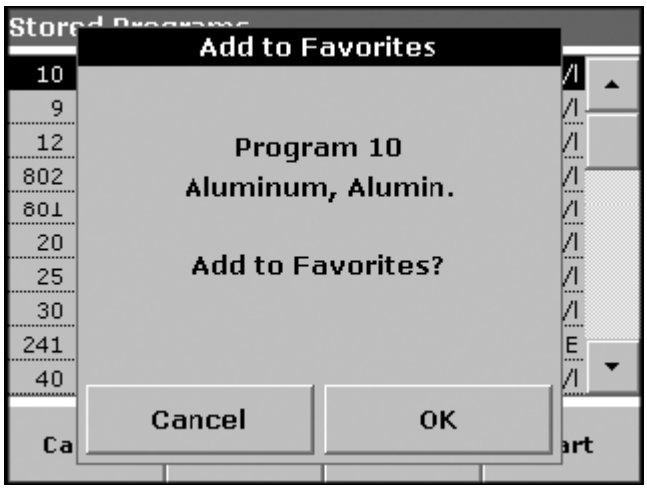
- <u>Setting the General Purpose Timer</u> - A general purpose timer is also available in many programs.
  - Press the **TIMER** icon, and select General Timer.
  - Enter the length of the timed interval and press **OK**.



- Press OK to start the timer. The timer will beep when the timed interval ends.

- Adding Stored Programs to the Favorite Programs List - Frequently used programs can be added to a favorite list. Use the procedure below to establish a favorites list for the FDPMU target analytes.

    - From the Main Menu, press **STORED PROGRAMS**. The Stored Programs list will appear.
    - Highlight the selection or press **SELECT BY NUMBER** to search for the program by number.
    - Press **PROGRAM OPTIONS>ADD TO FAVORITES** and press OK to confirm.



    - The program can now be selected from **FAVORITE PROGRAMS** on the Main Menu.

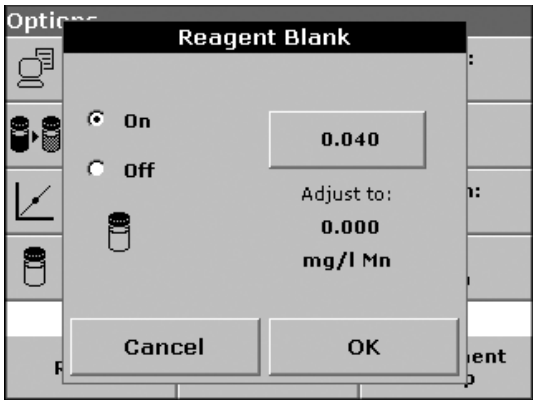
- Reagent Blanks – Upon initial instrument setup and whenever a new lot of reagents is opened, the operator must run reagent blanks. A reagent blank consists of de-ionized water (or some kind of reagent (laboratory) grade water) with the reagents used in the analysis added. The reagent blank detects impurities in the reagent lot that add color to the analysis and is seen by the instrument. Once the value of the reagent blank is established, it can be subtracted from subsequent analyses to yield a more precise result.
    - Running a Reagent Blank

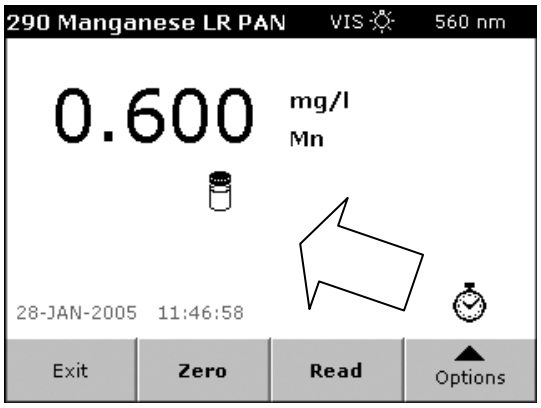
    1) Prepare the Test/Method as specified in the procedure. Instead of a sample, de-ionized water is used to determine the reagent blank value.
    2) Select the test. If required by the procedure, place the zero solution in the cell compartment. Close the compartment and press **ZERO**.
    3) Place the prepared cell in the cell compartment and close the cell compartment. Press **READ**. The result is displayed. Repeat this process two (2) more times and average the three (3) readings. If the averages is less than 0.005 it can be ignored. If the average is greater than 0.005, do the following:
       (a) Press **OPTIONS>MORE> REAGENT BLANK**.
       (b) Select On to activate the Reagent Blank function. The

concentration shown on the key is the most recent measured value and will be subtracted from subsequent measurements. To use this value for more analyses, press **OK**. If the measurement does not need to be saved, press the key to the right, and use the alphanumeric keypad to enter an average reagent blank value and press **OK** to confirm.

(c) Record the values for use on subsequent measurements using this lot of reagents.

Note: The Reagent Blank icon is shown in the result display.





6. Analysis of Samples

- Analytical Methods – Analytical methods of all DR 5000 target analytes are provided in Appendix A.
- Preservation - Samples that will not be analyzed within 24 hours of collection must be preserved. Preservation requirements per target analyte and the total volume of water that must be collected to complete all analyses and minimal quality assurance testing are listed in Table 6 below. Make sure the volume of the preservative does not affect the analysis. **The pH of preserved samples must be readjusted prior to analysis.** Adjusting the sample to within a pH range of 6 – 8 is appropriate for most analyses.

Table 6 - Preservation Requirements by Target Analyte

Preservative	Target Analyte	Minimum water Volume
2ml/liter Nitric Acid	Barium Cadmium Chromium (total) Copper Lead Selenium	1.0 Liter
2ml/liter Sulfuric Acid	Arsenic Nitrate	1.0 Liter
10ml/liter Hydrochloric Acid (no head space)	Mercury	1.0 Liter
4 ml/liter Sodium Hydroxide	Cyanide	250 mL
Chill to 4°C (no head space, warm to room temperature prior to analysis)	Alachlor Atrazine Color Fluoride Nitrite Trihalomethanes (total) Chloride Sulfate Volatile organic compounds (GC/MS)	1.0 Liter

- Correcting for Volume Additions - If you use a large volume of preservative, you must correct for the volume of preservative added.
    - This correction is made as follows:

      1) Determine the volume of initial sample, the volume of acid and base added, and the total or final volume of the sample.
      2) Divide the total volume by the initial volume of sample.
      3) Multiply the test result by this factor.

    - Sample Volume Correction - A one-liter sample was preserved with 2 mL of nitric acid. It was neutralized with 5 mL of 5N sodium hydroxide. The result of the analysis procedure was 10.00 mg/L.

What is the volume correction factor and correct result?

1) Total Volume = 1000 mL+2 mL+5 mL = 1007 mL
2) 1007 ÷ 1000 = 1.007 = volume correction factor, thus
3) 10.0 mg/L × 1.007 = 10.07 mg/L = correct result

- Method Notes

1. Apparent color is what the eye sees and directly affects the aesthetics of the water. Delete the filtration steps for the apparent color analysis
2. Due to the upper limit of detection of the Sulfate Method, it will typically be necessary to perform a 1:10 dilution prior to running the analysis.
3. Magnesium testing is done via the subtraction method. Analysis for total hardness and calcium hardness are run independently. The result of the calcium hardness test is subtracted from the result of the total hardness test to yield the concentration of magnesium in the sample.
4. Distillation is no longer required for any FDPMU water quality analysis method.
5. Total Dissolved Solids are tested using the HQ40d Dual-Input pH/Conductivity Meter (on older apparatus sets, TDS is tested using the sensION 156 Portable pH/Conductivity Meter).
6. Turbidity is tested by the P2100 Turbidimeter.

7. Sample Dilution

- Ten (10) mL and twenty-five (25) mL are the sample volumes used for most colorimetric tests. If a constituent is over-range, the color developed in the sample may be too intense to be measured. In addition, unexpected colors may develop in some tests if interfering substances are present. In both cases, samples can be analyzed accurately if a dilution is performed.
  - To dilute the sample, pipette the appropriate volume of sample for the desired dilution from Table 7 or Table 8 below into a clean volumetric flask. ***A 1 in 10 dilution will be effective in most circumstances***. Fill the flask to the desired volume (25 mL or 10 mL) with de-ionized water as indicated in the tables. Swirl the diluted sample to mix.
  - Use the diluted sample to running the analysis. Upon completing the analysis, multiply the result by the number in column 4 of the table. The concentration of the constituent in the sample is equal to the diluted sample reading times the multiplication factor.

Table 7 - Sample Dilutions for 25 mL Sample Volumes			
Dilution Factor	**Volume of Sample (mL)**	**Volume of de-ionized water to bring sample volume to 25 mL**	**Multiply result by:**
2	12.5	12.5	2
10	2.5	22.5	10
100	0.25	24.75	100

Table 8 - Sample Dilutions for 10 mL Sample Volumes			
Dilution Factor	**Volume of Sample (mL)**	**Volume of de-ionized water to bring sample volume to 10 mL**	**Multiply result by:**
2	5	5	2
10	1	9	10
100	0.01	9.99	100

8. Quality Assurance/Quality Control (QA/QC)

- Prior to and/or between deployments - All designated operators shall participate in Navy & Marine Corps Public Health Center Proficiency In Testing Quality Assurance Program (PITQAP) rounds for the DR 5000.

- During Deployments -

    - To the extent possible, all designated operators shall participate in Navy & Marine Corps Public Health Center PITQAP rounds for the DR 5000.

    - In addition, quality control testing shall be accomplished by standard addition per the Hach DR 5000 User Manual as follows:

        1) In the absence of actual samples, analysis of de-ionized water, spiked by standard addition, shall be completed for 5 selected target analytes each week. As there are approximately twenty DR 5000 target analytes, each of the methods should be tested at least once per month.
        2) When in receipt of samples, quality control testing shall be performed by standard addition on five randomly selected methods during the complete analysis. In addition, quality control testing shall be performed by standard addition whenever a result is within ±

20% of a Military Exposure Guideline, Tri-service Field Water Standard or USEPA Maximum Contaminant Level.

3) Results of QA/QC testing must be documented in each analytical report returned to a client and recorded in the unit's water quality analysis (WQA) log. Submit WQA logs to the Navy & Marine Corps Public health Center for archiving upon re-deployment.

9. Maintenance

- Cleaning Requirements -

  - Spectrophotometer
    1) Keep the surface of the instrument, the cell compartment, and all accessories clean and dry at all times. Splashes or spills on and in the instrument should be cleaned up immediately.
    2) Clean the enclosure, the cell compartment, and all accessories with a soft damp cloth. A mild soap solution can also be used. Dry the cleaned parts carefully with a soft cotton cloth.
  - Display - Clean the display with a soft, lint-free and oil-free cotton cloth. Diluted window cleaner can also be used.

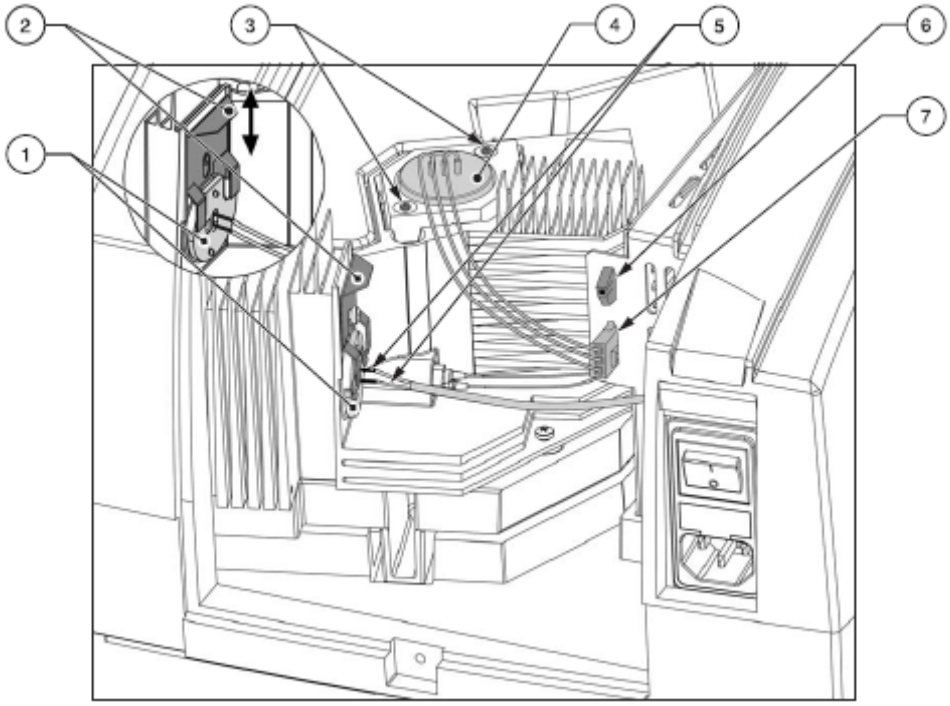
  - Glass Cells
    1) Clean glass cells with detergent and water.
    2) After cleaning, rinse the cells several times with tap water and then thoroughly with de-ionized water.

  Note: take care not to scratch or etch sample cells while cleaning! Glass cells that have been used for organic solvents (such as chloroform, benzene, toluene, etc.) must be rinsed with acetone before being treated with cleaning agents. In addition, another rinse with acetone is necessary as a final treatment step before the cells are dried.

- Lamp Replacement - The Lamp compartment is on the left side behind the display. The tungsten and deuterium (UV) lamp are installed in the lamp compartment. There is a fan on the back for cooling electric components within the compartment. The interior of the lamp compartment is depicted in the figure below.

CAUTION: Never look directly at an operating lamp without wearing UV protective eye glasses

Lamp Compartment



1. Tungsten Lamp	5. Contacts of Tungsten Lamp w/ Cable
2. Spring	6. Plug contacts for fan
3. Screws	7. Plug contacts for lamp
4. Deuterium lamp (lamp socket)	

- Changing the Tungsten Lamp -

  - Switch the instrument off and unplug the power cord.
  - Use a screwdriver to remove the cover from the back of the instrument (the screws may be slotted or phillips).
  - Place the cover and the attached fan carefully beside the instrument (take special care with the fan cable).
  - Push the spring (item 2, Figure 6) up and remove the tungsten lamp (item 1, Figure 5) from the lamp compartment.
  - Unplug both plug contacts (item 5, Figure 6) from the Tungsten lamp.
  - Push the plug contacts firmly onto the new Tungsten lamp.
  - Insert the tungsten lamp into the lamp compartment. Push the spring down.
  - Check that the spring and the lamp socket are positioned correctly.
  - Use a screwdriver to reinstall the back cover.
  - Plug in the power supply. The instrument is now ready for use.
  - Switch the instrument on.
  - Reset the Lamp History (refer to section 6.8.6 in the User Manual).

DANGER! Remove power from the instrument prior to changing the lamp. Wait until the lamp has cooled prior to replacement.

- Changing the Deuterium Lamp (UV)

    - Switch the instrument off. Unplug the power cord.
    - Use a screwdriver to remove the cover from the back of the instrument (the screws may be slotted or phillips).
    - Place the cover and the attached fan carefully beside the instrument (take special care with the fan cable).
    - Unplug the deuterium lamp (item 5, Figure 6) from the socket by
    - pushing down on the safety contact.
    - Use a screwdriver to unscrew the two fastening screws (item 3, Figure 6) (the screws may be slotted or phillips) from of the socket.
    - Holding the lamp socket, lift the deuterium lamp up and out of the lamp compartment (remove the complete unit, including the cable).
    - Carefully insert the new deuterium lamp into the lamp compartment.
    - Screw in both fastening screws until they are finger-tight.
    - Insert the deuterium lamp cable connector in the socket so that the safety contact clicks into place.
    - Use a screwdriver to reinstall the back cover.
    - Plug in the power supply. The instrument is now ready for use.
    - Switch the instrument on.
    - Reset the Lamp History, (refer to section 6.8.6 in the User Manual).

DANGER! Remove power from the instrument prior to changing the lamp. Wait until the lamp has cooled prior to replacement.

Important Note: Do not touch the glass envelope on the new lamp. If it is touched, clean with alcohol.

10. Trouble Shooting

Problem	Likely Cause	Solution
Absorbance > 3.5!	The measured absorbance exceeds 3.5.	Dilute the sample and repeat the measurement.
Concentration too high!	Calculated concentration is higher than 999999.	Dilute the sample and repeat the measurement.
Error **Clean Cuvette**	The cuvette is soiled or there are undissolved particles in the cuvette.	Clean the cuvette; allow the particles to settle.
Error **Please check the UV Lamp**	The lamp output is too low.	Check the lamp and replace it if necessary.
Error **Selfcheck stopped.** **Please remove the cuvette**	Self Check Test stops while starting the instrument.	Remove the vial. Press **OK**.
Hardware error	Electronic defect	Contact Technical Support.
Negative result!	The calculated result is negative.	Check the concentration of the sample.
No evaluation!	Error in the Test database.	Contact Technical Support.
Over calibration range	During a polygonal interpolation, the measured absorbance exceeds the calibration range of the test.	Dilute the sample and repeat the measurement.
Over measuring range	The measured absorbance is above the calibration range of the test.	Dilute the sample and repeat the measurement.
Please check the lamp	The lamp output is too low.	Check the lamp and replace it if necessary.
Please close the lid	Self Check Test stops while starting the instrument.	Close the lid. Press **START AGAIN**.
Temperature too high! **Lamps are off!**	The measured temperature is above the maximum limit.	Turn the instrument off **immediately** to cool down. Change the filter pad.
Under calibration range	During a polygonal interpolation, the measured absorbance is below the calibration range of the test.	Change the calibration range.
Under measuring range	The measured absorbance is below the calibration range of the test.	If possible, select a test with a lower measurement range or use a vial with a longer path length.

11. References

DR 5000 Spectrophotometer User Manual, January, 2008 Edition 2 (or current edition)

DR5000 Spectrophotometer Procedures Manual, May 2005, Edition 2 (or current edition)

USACHPPM Technical Guide 230, Chemical Exposure Guidelines for Deployed Military Personnel, Version 1.3 May 2003 with January 2004 Addendum (or current version)

Appendix A - DR 5000 Analytical Methods

Analyte	Method	Hach Program #
Alachlor	Method 10202	Immunoassay Method (450nm)
Arsenic	Method 28000	N/A
Atrazine	Method 10500	Immunoassay Method (450nm)
Barium	Method 8015	25
Cadmium	Method 8017	60
Chloride	Method 8113	70
Chromium, Total	Method 8024	100
Color, Apparent	Method 8025	User defined (455nm), see Method Note 1 on page 20 of the SOP.
Copper	Method 8026	135
Cyanide	Method 8027	160
Fluoride	Method 8029	190
Lead	Method 8317	283
Mercury	Method 10065	312
Nitrate `	Method 10020	344
Nitrite	Method 10019	345
Selenium	Method 8194	640
Sulfate	Method 8051	685, see Method Note 2 on page 20 of the SOP
Trihalomethanes	Method 10132	725
Hardness, Total	Method 8213	Digital Titration, see Method Note 3 on page 20 of the SOP.
Hardness, Calcium	Method 8204	Digital Titration, see Method Note 3 on page 20 of the SOP.

Alachlor

Method 10202

Immunoassay Method[1]

Scope and Application: For water

[1] This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

Test Preparation

This method analyzes for Alachlor in water. Sample calibrators and reagents are added to cuvettes coated with Alachlor-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 30 minutes for complete analysis. As many as 20 cuvettes (18 samples and 2 calibrators) can be run simultaneously.

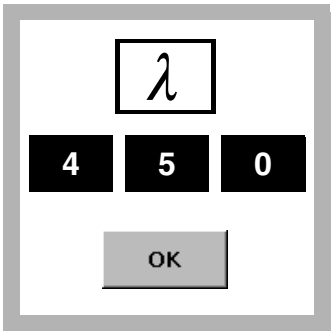
Before starting the test:

Read the entire procedure before starting. Identify and make ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.
Timing is critical; follow instructions carefully.
A consistent technique when mixing the cuvettes is critical to this test. The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack on page 4*. Cuvettes can be mixed individually, but test results may not be as consistent.
Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument.
Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
The cuvette rack is designed to be inverted with the cuvettes in place. This is especially helpful when running many samples at once; the cuvettes can remain in the rack and be processed together until they are read in the spectrophotometer.
Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Cuvettes are not reusable.
Protective nitrile gloves are recommended for this procedure.

Collect the following items	**Quantity**
Alachlor Reagent Set	1
Caps, flip spout	1
Marker, laboratory	1
Rack, for 1-cm Micro Cuvettes	1
Wipes, disposable	1
Pipet, TenSette®, 0.1–1.0 mL	1
Pipet tip for 19700-01, TenSette Pipet	1

Note: *Reorder information for consumables and replacement items is on page 7.*

Immunoassay for Water

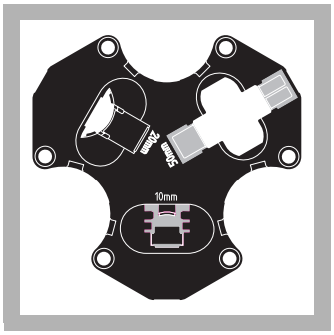


1. Press

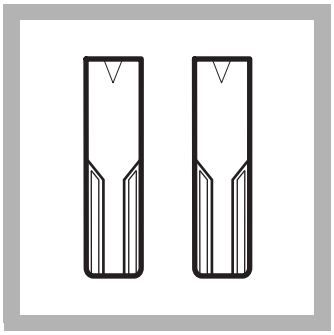
SINGLE WAVELENGTH

Press **OPTIONS** and the λ button. Type in

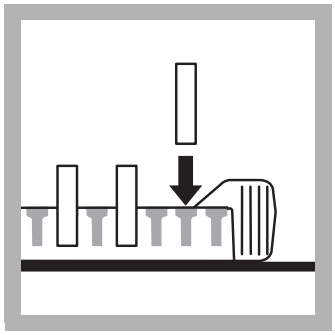
450 nm and press **OK.**



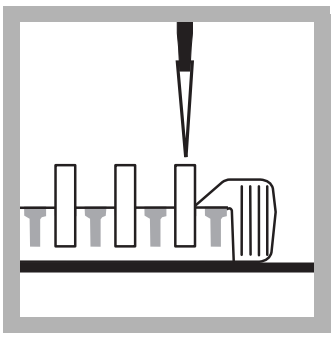
2. Insert the Multi-cell Adapter with the 10 mm square cell holder facing the user.



3. Label an Antibody Cuvette for each calibrator and each sample to be tested.

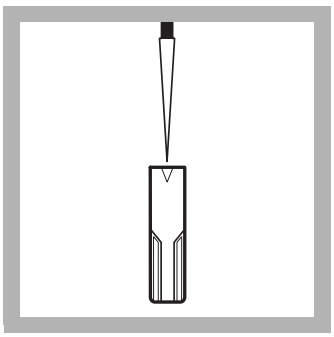


4. Insert the cuvettes into the rack snugly.



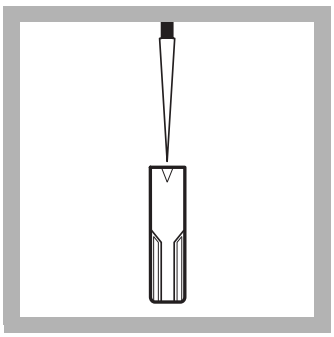
5. Pipet 0.5 mL of each calibrator into the appropriately labeled cuvette.

Use a new pipette tip for each calibrator.

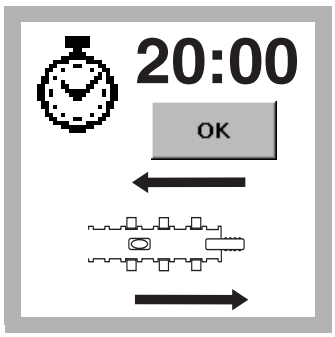


6. Pipet 0.5 mL of each sample to be tested into the appropriately labeled cuvette.

Use a new pipette tip for each sample.



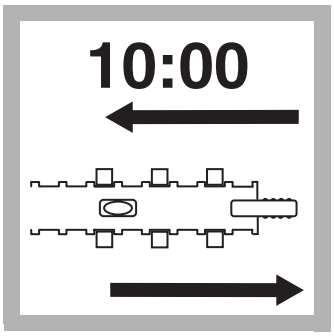
7. Immediately pipet 0.5 mL of Alachlor Enzyme Conjugate into each cuvette.



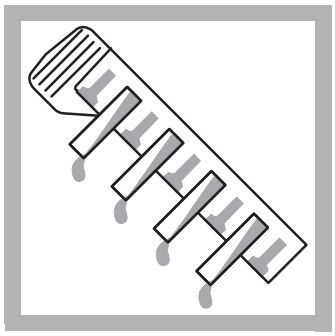
8. Press **OPTIONS**. Press **TIMER**. Enter 20:00 minutes and press **OK.**

A 20-minute reaction time will begin.

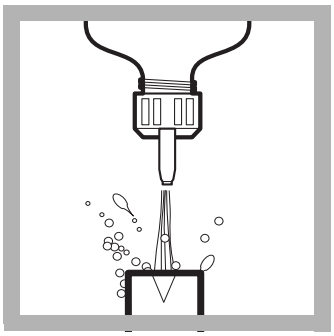
Immediately mix the contents of the cuvettes for 30 seconds using the technique described in Using the 1-cm MicroCuvette Rack on page 4.



9. After 10 minutes mix the contents of the rack for 30 seconds using the technique described in Using the 1-cm MicroCuvette Rack on page 4.



10. At the end of the 20-minute period, discard the contents of all the cuvettes into an appropriate waste container.

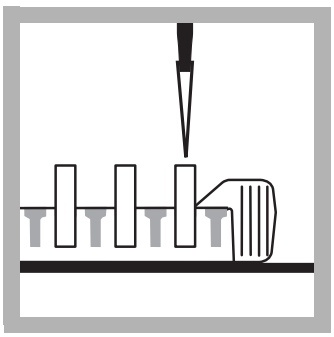


11. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Ensure that most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel.

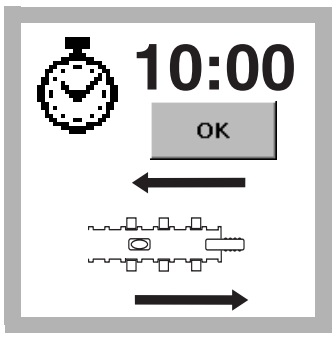
Color Development

Important Note: *Timing is critical. Follow instructions carefully.*



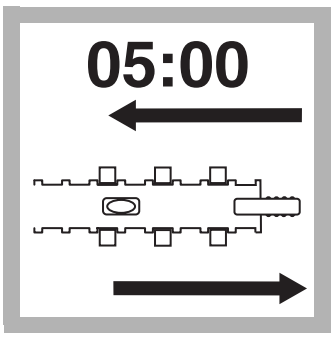
12. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Use a new pipette tip for each cuvette.



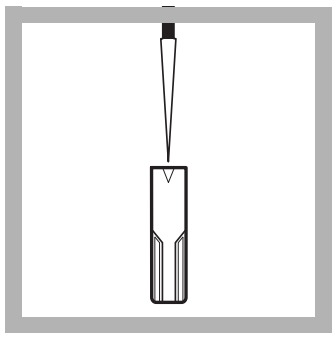
13. Press **OPTIONS**. Press **TIMER**. Enter 10:00 minutes and press **OK.**

A reaction period will begin. Mix, using the instructions on page *4*.



14. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

Solutions will turn blue in some or all of the cuvettes.



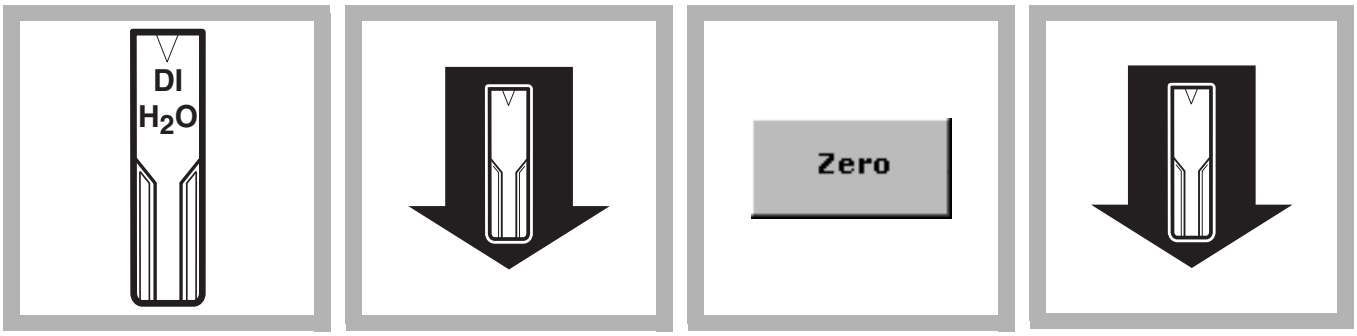
15. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in step 12.

Slide the rack for 20 seconds (Using the 1-cm MicroCuvette Rack on page 4.)

Blue solutions will turn yellow with the addition of the Stop Solution.

Use the same pipette tip repeatedly for this step.

Measuring the Color



16. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.

17. Insert the filled zeroing cuvette into the cell holder—arrow pointing towards the front of the instrument.

Orient the arrow in the same direction for all cuvettes.

18. Press **ZERO**.

The display will show:

0.000 Abs

19. Insert the first calibrator into the cell holder.

The display will give an absorbance reading. Record the results for each calibrator and sample.

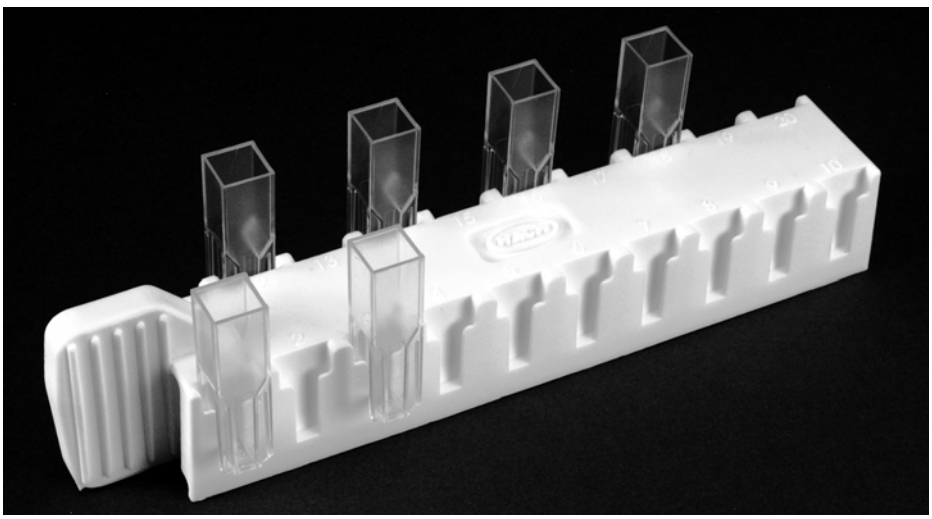
Repeat this step for all remaining calibrators and samples.

See Interpreting and Reporting Results for help with interpretation of results.

Using the 1-cm MicroCuvette Rack

The MicroCuvette rack (Figure 1) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

Figure 1 The 1-cm MicroCuvette Rack



Loading the Rack—The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and insert all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing—Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and Reporting Results

There is an inverse relationship between the concentration of Alachlor and the reading. In other words, the higher the reading, the lower the concentration of Alachlor.

Table 1 Relative Alachlor Concentration

If the sample reading is...	the sample Alachlor Concentration is...
…less than calibrator reading	…greater than the calibrator concentration
…greater than calibrator reading	…less than the calibrator concentration

Example
Readings:

0.1 ppb Alachlor Calibrator: **0.475 Abs**

0.5 ppb Alachlor Calibrator: **0.245 Abs**

Sample #1: **0.140 Abs**

Sample #2: **0.300 Abs**

Sample #3: **0.550 Abs**

Interpretation
Sample #1—Sample reading is less than the readings for both calibrators. Therefore the sample concentration of Alachlor is greater than both 0.1 ppb and 0.5 ppb Alachlor.

Sample #2—Sample reading is between the readings for the 0.1 ppb and 0.5 ppb Alachlor calibrators. Therefore the sample concentration of Alachlor is between 0.1 ppb and 0.5 ppb.

Sample #3—Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of Alachlor is less than both 0.5 ppb and 0.1 ppb.

Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
- Keep the foil pouch containing the Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The Alachlor immunoassay test cannot differentiate between certain herbicides and metabolites, but it detects their presence to differing degrees. Table 2 shows the required concentration for selected chemicals.

Table 2 Required Concentration for Selected Chemicals

Compound	Concentration to give a positive response of 0.1 ppb Alachlor	Concentration to give a positive response of 0.5 ppb Alachlor
Acetochlor	0.45 ppb	4 ppb
Butachlor	0.09 ppm	1 ppm
2 Chloro-2',6'-Diethylacetaniline	0.030 ppm	2 ppm
Metolachlor	0.085 ppm	2 ppm
2,6-Diethylaniline	0.3 ppm	9 ppm
Propachlor	0.72 ppb	12 ppb

Sample Collection and Storage

Table 3 Compounds not detectable at 10,000 ppb

Atrazine	2, 4-D
Aldicarb	Chlorpyirfos
Diazoton	Carbendazim
Carbofuran	

Collect samples in a clean glass bottle. Do not pre-rinse the bottle with the sample. If the sample cannot be analyzed immediately, store the sample at 4 °C. Samples may be kept for as long as 14 days. Warm the samples to room temperature before analysis.

Diluting Water Samples

Other levels of Alachlor can be tested by diluting the sample and comparing the results to the 0.1 ppb Calibrator. Select the appropriate sample volume from Table 4, place it in a graduated mixing cylinder, and dilute it to 50 mL with deionized water.

Table 4 Sample Volume and Concentration

mL Sample	Representative Concentration using 0.1 ppb Calibrator
0.5	10 ppb
1.0	5 ppb
2.5	2 ppb
5.0	1 ppb

Example:
Dilute 2.5 mL of sample to 50 mL with deionized water. Run the diluted sample in the procedure along with the 0.1 ppb calibrator. If the absorbance of the diluted sample is less than the 0.1 ppb calibrator, the concentration of the original sample is greater than 2 ppb.

Summary of Method

Immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Alachlor-specific antibodies, attached to the walls of plastic cuvettes, selectively bind and remove Alachlor from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and Alachlor compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by Alachlor and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of Alachlor in the sample. The resulting color is then compared with a calibrator to determine whether the Alachlor concentration in the sample is greater or less than the threshold levels. Test results are measured at 450 nm.

Consumables and Replacement Items

Required Reagents

Description	Unit	Cat. No.
Alachlor Reagent Set[1]	20 cuvettes	28130-00

[1] Immunoassay components are manufactured by Beacon Analytical Systems, Inc.

Required Apparatus

Description	Unit	Cat. No.
Caps, flip spout	2/pkg	25818-02
Marker, laboratory	each	20920-00
Pipet, TenSette®, Pipet, 0.1–1.0 mL	each	19700-01
Pipet Tips, for TenSette Pipet 19700-01	1000/pkg	21856-28
Rack, for 1-cm Micro Cuvettes	each	48799-00
Wipes, disposable	box	20970-00

Optional Reagents and Apparatus

Description	Unit	Cat. No.
Glasses, Safety	each	27568-00
Gloves, Disposable, Nitrile, Medium[1]	each	25505-02
Pipet Tips, for TenSette Pipet 19700-01	50/pkg	21856-96

[1] Other sizes available.

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Updated April 05 Edition 1

Arsenic Test Kit 0–500 ppb (0, 10, 30, 50, 70, 300, 500 ppb) 28000-88

WARNING: Hydrogen and arsine gases are generated during the test. Work in a well-ventilated area away from open flames and other sources of ignition. Review the Material Safety Data Sheets before handling any chemicals.

Scope and Application: For natural waters, drinking water, and groundwater

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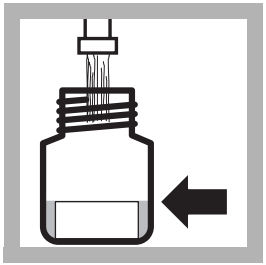
Introduction

Hach's new Arsenic test kit provides a simple, effective way to test for arsenic in the range of 0–500 ppb. The visual comparison test is ideal for use almost anywhere that trace amounts of total inorganic arsenic must be quantified. This new kit uses safe, easy-to-handle reagents packaged in unit doses, with a test strip to determine the final result. Up to 5 mg/L hydrogen sulfide in the sample can be tolerated. The design of the apparatus offers increased sensitivity (down to 10 ppb) and also minimizes exposure to arsine gas.

Tips and Techniques

1. Do not expose the **reacted** test strips to direct sunlight. The reaction products are photosensitive and will tend to darken, which may cause difficulty in color matching.
2. At no time should the solution in the reaction vessel come into direct contact with the test strip. The test strip reacts with gases released from the chemical reaction, not with the solution in the reaction vessel.
3. It is **critical** that the pad on the test strip **face downward**, centered over the hole in the black cap. If the placement of the test strip is incorrect, the generated gases may not contact the pad correctly and the final reading may be low.
4. Two reaction vessels and two black caps are provided to allow for the simultaneous analysis of two samples.

Procedure



English

1. Lift the flap on the black cap and **slide** a test strip into the groove so that the reactive pad faces the small opening and completely covers it; secure by pressing the flap back in place.

2. Fill the reaction vessel with sample water to the fill line (50 mL).

3. Add the contents of 1 Reagent #1 powder pillow to the sample and swirl to dissolve.

4. Add the contents of 1 Reagent #2 powder pillow to the sample and swirl to dissolve.

Note: *Solution may be cloudy at this point.*

Español

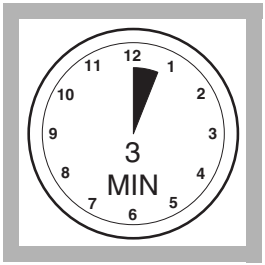
1. Levante la trampilla encima del tapón negro y deslize una tira de prueba en la ranura, cuidando que la almohadilla reactiva mire hacia la abertura y la cubra completamente; cierre la trampilla presionándola.

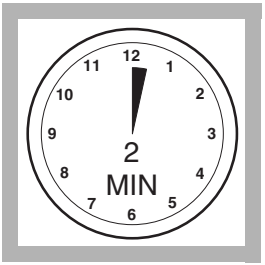
2. Llene el frasco de reacción con la muestra de agua hasta la marca (50 mL).

3. Agregue el contenido de 1 cápsula de polvo Reactivo #1 a la muestra y revolver para disolver.

4. Agregue el contenido de 1 cápsula de polvo Reactivo #2 a la muestra y revuelva para disolver.

Nota: *La solución se verá opaca en este punto.*





English

5. Wait at least 3 minutes.

6. Add the contents of 1 Reagent #3 powder pillow to the sample and swirl to mix.

Note: *Not all of the powder will dissolve.*

7. Wait at least 2 minutes and swirl again to mix.

8. Using the plastic scoop, add 1 level scoop of Reagent #4 to the sample and swirl to mix.

Note: *Most of the powder will dissolve at this time.*

Español

5. Espere por lo menos 3 minutos.

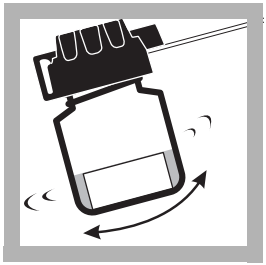
6. Agregue el contenido de 1 cápsula de polvo Reactivo #3 a la muestra y revuelva para disolver.

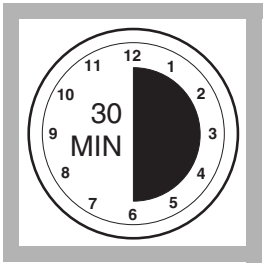
Nota: *No todo el polvo entrará en solución.*

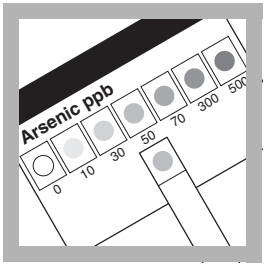
7. Espere por lo menos 2 minutos y revuelva de nuevo para mezclar.

8. Empleando la cuchara plástica, agregue 1 cucharada rasa de Reactivo #4 a la muestra, y revuelva para mezclar.

Nota: *Ahora se disolverá la mayor parte del polvo.*







English

9. Add the contents of 1 Reagent #5 powder pillow to the sample.

10. Immediately attach the black cap, with the test strip inserted, to the reaction vessel. **Do not shake or invert!**

Swirl to mix. Do not allow sample to contact the test strip pad.

11. Allow vessel to react for 30 minutes, but no more than 35 minutes; swirl twice during the reaction period.

12. Remove the test strip and immediately compare the developed color to the chart on the test strip bottle.

Note: *For best results, read the strip outdoors in a shady place. Direct sunlight will change the color of the strip.*

Español

9. Agregue el contenido de 1 cápsula de polvo Reactivo #5 a la muestra.

10. Inmediatamente vuelva a tapar el frasco de reacción con el tapón negro con la tira de prueba inserta. **¡No sacuda ni invierta el frasco!**

Revuelva para mezclar. No permita que muestra tenga contcto con tira de prueba.

11. Deje que la reacción proceda por 30 minutos, pero no más de 35 minutos; revuelva 2 veces durante el período de la reacción.

12. Retire la tira de prueba y compare inmediatamente el color revelado con la carta de color pegada al recipiente de las tiras de prueba.

Note: *Para lograr resultados más exactos, lea la tira de prueba afuera, pero en un sitio sombreado. La luz directa del sol alterará el color de la tira.*

Interferences

The following were found to interfere:

Ion or Substance	Concentration
Sulfide	> 5 ppm
Selenium	> 1 ppm
Antimony	> 250 µg/L
Tellurium	Likely to interfere, but not tested

The following did not interfere at the levels tested:

Ion or Substance	Concentration
Hardness	1000 ppm as $CaCO_3$
Alkalinity	1000 ppm as $CaCO_3$
Iron	10 ppm
Temperature	10 to 40 °C

Other interferences are unlikely.

Summary of Method

Hydrogen sulfide is first oxidized to sulfate to prevent interference, and the oxidizing environment is then neutralized. Sulfamic acid and powdered zinc react to create strong reducing conditions in which inorganic arsenic is reduced to arsine gas (AsH_3). The arsine gas then reacts with mercuric bromide in the test strip to form mixed arsenic/mercury halogenides that discolor the test strip. The color ranges from yellow through tan to brown, depending on the concentration.

Organic Arsenic

Organic arsenic represents a small proportion of the arsenic in most systems. The instructions, as written for this test, are designed to detect inorganic arsenic. Organic arsenic compounds, such as dimethylarsenic acid, are not detected. To quantify inorganic and organic arsenic (total arsenic) with this kit, the following modification is needed: Collect 50 mL of sample in a glass beaker. Add the first two reagents according to the instructions. Place the beaker in a boiling water bath for 30 minutes. Remove the beaker from the water bath and transfer the contents to the reaction vessel. Allow the sample to cool to room temperature. Complete the procedure, beginning with step 6.

Required Reagents

Description	Unit	Cat. No.
Arsenic Test Kit Reagent Set	each	27999-00
Includes: Arsenic Test Strips and Arsenic Reagents #1 – #5		
Arsenic Test Strips	100/pkg	*
Arsenic Reagent #1, Powder Pillows	100/pkg	*
Arsenic Reagent #2, Powder Pillows	100/pkg	*
Arsenic Reagent #3, Powder Pillows	100/pkg	*
Arsenic Reagent #4	250 g	*
Arsenic Reagent #5, Powder Pillows	100/pkg	*
Cap, Santoprene	2/pkg	49348-00
Reaction Vessel, 50-mL fill line	2/pkg	28002-00
Scoop, 2 g, for 454-29	each	27998-00

* These items are not sold separately. Please order the complete reagent set (Cat. No. 27999-00) as a replacement.

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jb/dk/rb 06/00 1ed

Atrazine

Method 10050

Immunoassay Method[1]

Scope and Application: For water

[1] This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

Test Preparation

This method analyzes for Atrazine in water. Sample calibrators and reagents are added to cuvettes coated with Atrazine-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 30 minutes for complete analysis. As many as 20 cuvettes (18 samples and 2 calibrators) can be run simultaneously.

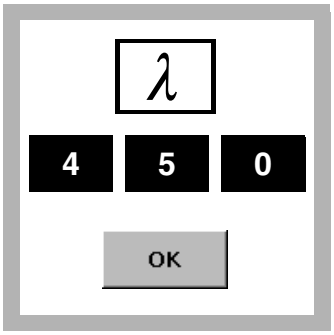
Before starting the test:

Read the entire procedure before starting. Identify and make ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.
Timing is critical; follow instructions carefully.
A consistent technique when mixing the cuvettes is critical to this test. The best results come from using the cuvette rack and mixing as described in Using the 1-cm MicroCuvette Rack on page 4. Cuvettes can be mixed individually, but test results may not be as consistent.
Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument.
Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
The cuvette rack is designed to be inverted with the cuvettes in place. This is especially helpful when running many samples at once; the cuvettes can remain in the rack and be processed together until they are read in the spectrophotometer.
Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Cuvettes are not reusable.
Protective nitrile gloves are recommended for this procedure.

Collect the following items:	**Quantity**
Atrazine Reagent Set	1
Caps, flip spout	1
Marker, laboratory	1
Rack, for 1-cm Micro Cuvettes	1
Wipes, disposable	1
Pipet, TenSette®, 0.1–1.0 mL	1
Pipet Tips, for TenSette Pipet 19700-01	1

Note: *Reorder information for consumables and replacement items is on page 7.*

Immunoassay for Water

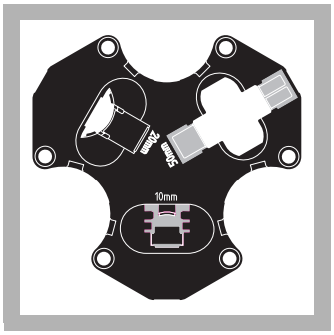


1. Press

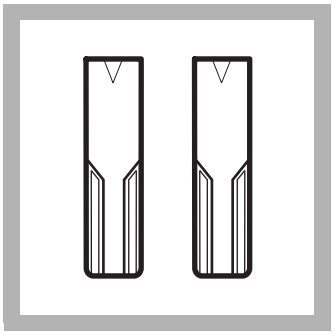
SINGLE WAVELENGTH

Press **OPTIONS** and press the λ button. Type in

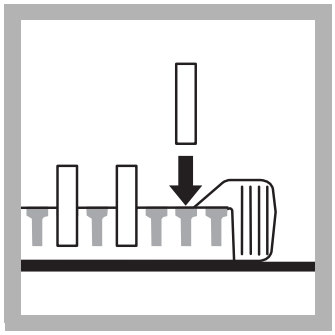
450 nm and press **OK.**



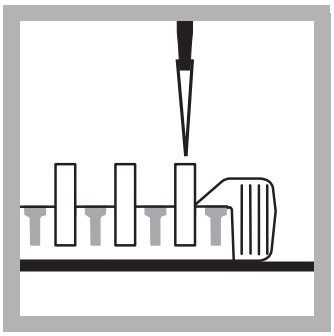
2. Insert the Multi-cell Adapter with the 10 mm square cell holder facing the user.



3. Label an Antibody Cuvette for each calibrator and each sample to be tested.

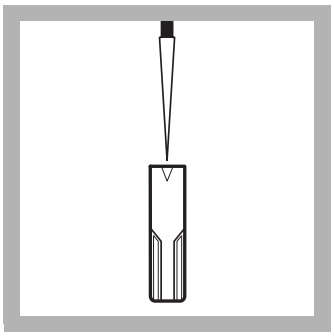


4. Insert the cuvettes into the rack snugly.



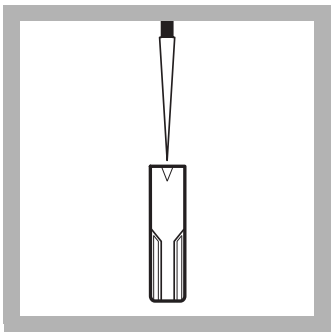
5. Pipet 0.5 mL of each calibrator into the appropriately labeled cuvette.

Use a new pipette tip for each calibrator.

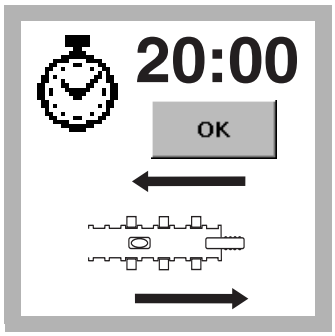


6. Pipet 0.5 mL of each sample to be tested into the appropriately labeled cuvette.

Use a new pipette tip for each sample.



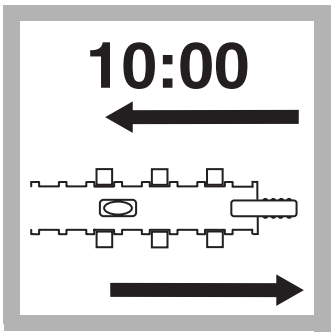
7. Immediately pipet 0.5 mL of Atrazine Enzyme Conjugate into each cuvette.



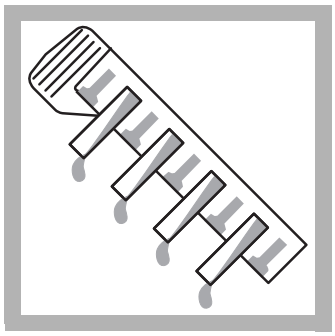
8. Press **OPTIONS**. Press **TIMER**. Enter 20:00 minutes and press **OK.**

A 20-minute reaction time will begin.

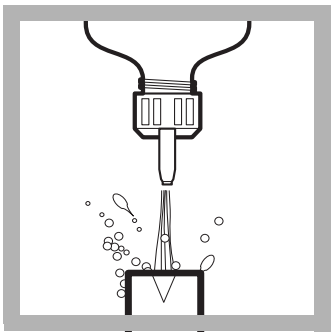
Immediately mix the contents of the cuvettes for 30 seconds (Using the 1-cm MicroCuvette Rack on page 4.)



9. After 10 minutes mix the contents of the rack for 30 seconds (Using the 1-cm MicroCuvette Rack on page 4.)



10. At the end of the 20-minute period, discard the contents of all the cuvettes into an appropriate waste container.

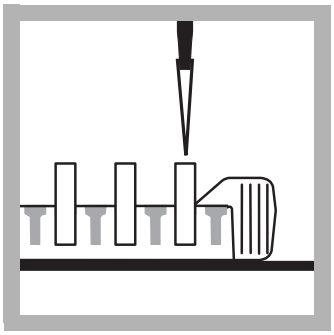


11. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Ensure that most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel.

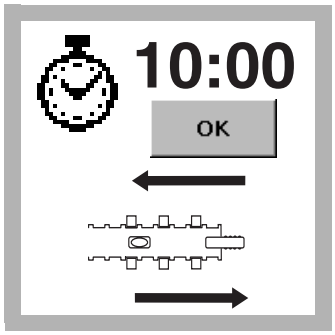
Color Development

Important Note: *Timing is critical. Follow instructions carefully.*



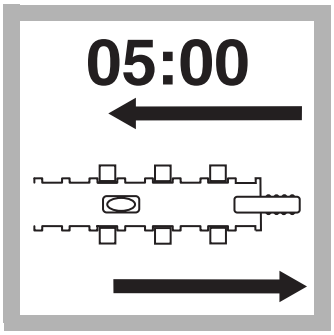
12. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Use a new pipette tip for each cuvette.



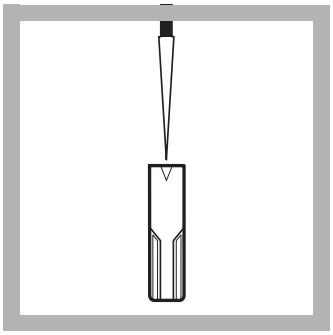
13. Press **OPTIONS**. Press **TIMER**. Enter 10:00 minutes and press **OK.**

A reaction period will begin. Mix, using the instructions on page 4.



14. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

Solutions will turn blue in some or all of the cuvettes.

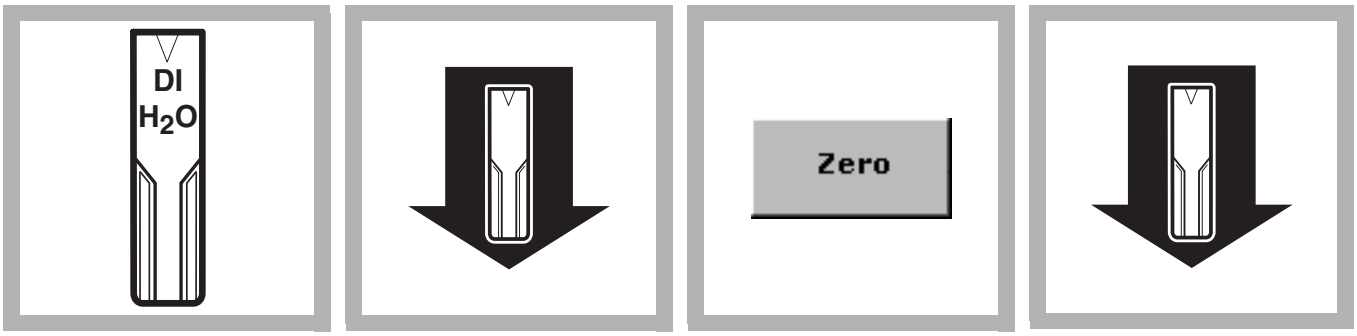


15. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in step 12. Use the same pipette tip repeatedly for this step.

Slide the rack for 20 seconds (Using the 1-cm MicroCuvette Rack on page 4.)

Blue solutions will turn yellow with the addition of the Stop Solution.

Measuring the Color



16. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.

17. Insert the filled zeroing cuvette into the cell holder—arrow pointing towards the front of the instrument.

Orient the arrow in the same direction for all cuvettes.

18. Press **ZERO**.

The display will show:

0.000 Abs

19. Insert the first calibrator into the cell holder

The display will give an absorbance reading. Record the results for each calibrator and sample.

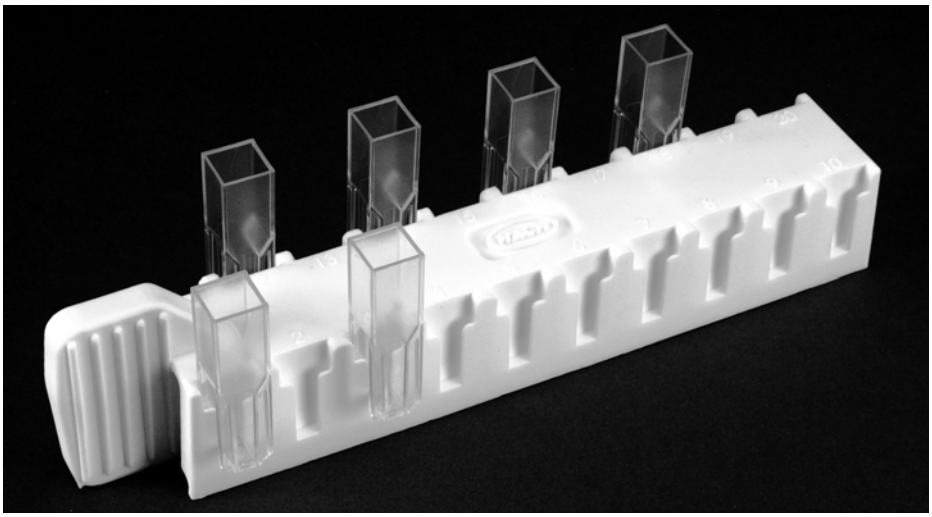
Repeat this step for all remaining calibrators and samples.

See Interpreting and Reporting Results on page 5 for help with interpretation of results.

Using the 1-cm MicroCuvette Rack

The MicroCuvette rack (Figure 1) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

Figure 1 The 1-cm MicroCuvette Rack



Loading the Rack—The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and insert all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing—Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and Reporting Results

There is an inverse relationship between the concentration of Atrazine and the reading. In other words, the higher the reading, the lower the concentration of Atrazine.

Table 1 Relative Atrazine Concentration

If the sample reading is...	the sample Atrazine Concentration is...
…less than calibrator reading	…greater than the calibrator concentration
…greater than calibrator reading	…less than the calibrator concentration

Example
Readings:

0.5 ppb Atrazine Calibrator: **0.475 Abs**

3.0 ppb Atrazine Calibrator: **0.245 Abs**

Sample #1: **0.140 Abs**

Sample #2: **0.300 Abs**

Sample #3: **0.550 Abs**

Interpretation
Sample #1—Sample reading is less than the readings for both calibrators. Therefore the sample concentration of Atrazine is greater than both 0.5 ppb and 3.0 ppb Atrazine.

Sample #2—Sample reading is between the readings for the 0.5 ppb and 3.0 ppb Atrazine calibrators. Therefore the sample concentration of Atrazine is between 0.5 ppb and 3.0 ppb.

Sample #3—Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of Atrazine is less than both 3.0 ppb and 0.5 ppb.

Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
- Keep the foil pouch containing the Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The Atrazine immunoassay test cannot differentiate between certain triazines and metabolites, but it detects their presence to differing degrees. Table 2 shows the required concentration for selected chemicals.

Table 2 Required Concentrations for Selected Chemicals

Compound	Concentration to give a positive result at 3 ppb (in ppb)
Ametryne	1
Atrazine	3
Atrazine, de-ethylated	115
Atrazine, de-isopropyl	714
Cyanazine	460
Cyromazine	1200
Prometon	8
Prometryne	0.7
Propazine	2.3
Simetryne	5.4
Simazine	37
Terbuthylazine	91
Terbutryne	8.3

Table 3 Compounds not detectable at 10,000 ppb

Alachlor	2, 4-D
Aldicarb	Diaminoatrazine
Carbendazim	Melamine
Carbofuran	Metaolachlor

Sample Collection and Storage

Collect samples in a clean glass bottle. Do not pre-rinse the bottle with the sample. If the sample cannot be analyzed immediately, store the sample at 4 °C. Samples may be kept for as long as 14 days. Warm the samples to room temperature before analysis.

Summary of Method

Immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Atrazine-specific antibodies, attached to the walls of plastic cuvettes, selectively bind and remove Atrazine from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and Atrazine compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by Atrazine and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of Atrazine in the sample. The resulting color is then compared with a calibrator to determine whether the Atrazine concentration in the sample is greater or less than the threshold levels. Test results are measured at 450 nm.

Consumables and Replacement Items

Required Reagents

Description	Unit	Cat. No.
Atrazine Reagent Set[1]	20 cuvettes	27627-00

[1] Immunoassay components are manufactured by Beacon Analytical Systems, Inc.

Required Apparatus

Description	Unit	Cat. No.
Caps, flip spout	2/pkg	25818-02
Marker, laboratory	each	20920-00
Pipet, TenSette®, 0.1–1.0 mL	each	19700-01
Pipet Tips, for TenSette Pipet 19700-01	1000/pkg	21856-28
Rack, for 1-cm Micro Cuvettes	each	48799-00
Wipes, disposable	box	20970-00

Optional Reagents and Apparatus

Description	Unit	Cat. No.
Atrazine Reagent Set	100 cuvettes	27627-10
Glasses, Safety	each	27568-00
Gloves, Disposable, Nitrile, Medium[1]		25505-02
Pipet Tips, for TenSette Pipet 19700-01	50/pkg	21856-96

[1] Other sizes are available.

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Updated April 05 Edition 1

Barium

Method 8014

Turbidimetric Method*

Powder Pillows or AccuVac® Ampuls

(1 to 100 mg/L)

Scope and Application: For water, wastewater, oil-field water, and seawater

* Adapted from Snell and Snell, *Colorimetric Methods of Analysis, Vol. II*, 769 (1959).

Tips and Techniques

- Perform a standard curve adjustment or a new calibration for each new lot of reagent. See *Standard Solutions* and *Calibration Standard Preparation*.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Filter highly colored or turbid water samples using a funnel (Cat. No. 1083-67) and filter paper (Cat. No. 1894-57). Large amounts of color or turbidity will interfere and cause high readings.
- Immediately after each test, clean the sample cell with soap, water, and a brush to prevent a film of barium from forming inside the sample cell.

Powder Pillows — Method 8014

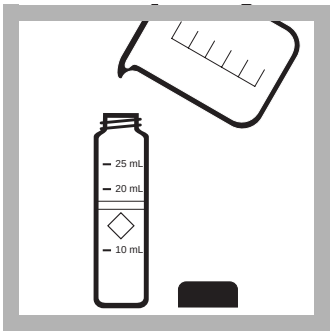
1. Touch

Hach Programs.

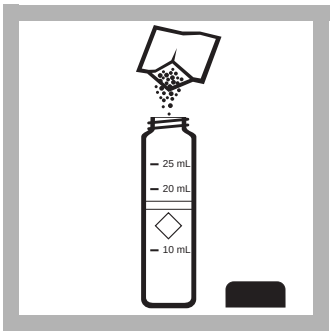
Select program

20 Barium

Touch **Start**.



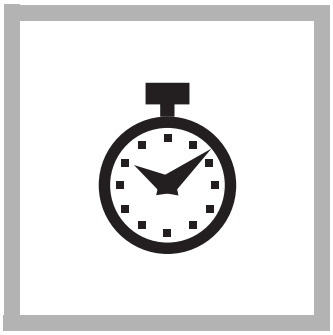
2. Fill a round sample cell with 25 mL of sample.



3. Add the contents of one BariVer® 4 Barium Reagent Powder Pillow to the cell (this is the prepared sample).

Cap and invert to mix.

Note: *If barium is present, a white turbidity will develop.*

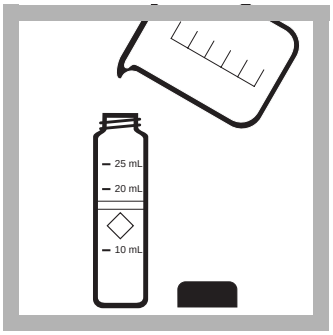


4. Touch the timer icon.

Touch **OK**.

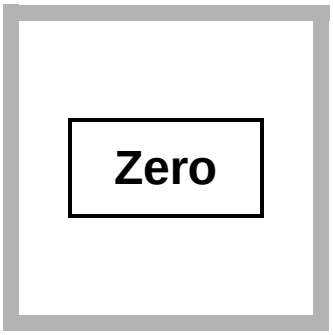
A five-minute reaction period will begin.

Do not disturb the sample during the reaction period.



5. Fill another sample cell with 25 mL of sample (this is the blank).

6. When the timer beeps, wipe the blank and place it into the cell holder.



7. Touch **Zero**.

The display will show:

0 mg/L Ba^{2+}

8. Within 10 minutes after the timer beep, wipe the prepared sample and place it into the cell holder.

Results will appear in mg/L Ba^{2+}.

AccuVac Ampul — Method 8014

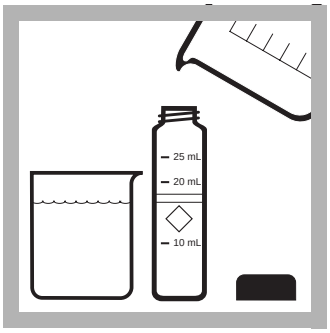
1. Touch

Hach Programs.

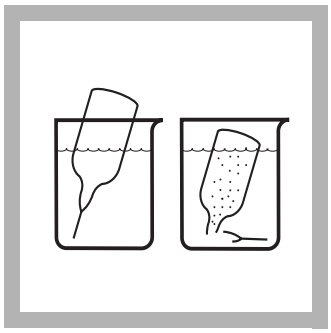
Select program

25 Barium, AV

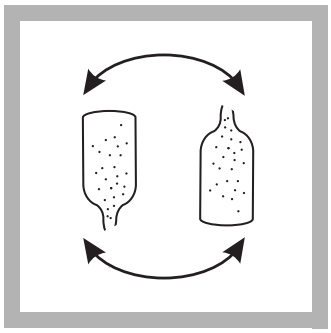
Touch **Start**.



2. Fill a round sample cell with 25-mL of sample (the blank). Collect at least 40 mL of sample in a 50-mL beaker.

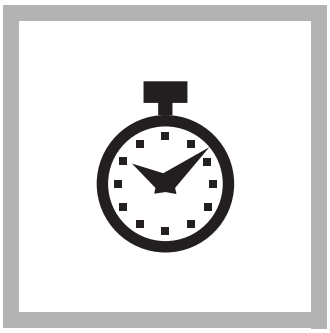


3. Fill a barium AccuVac® Ampul with sample. (This is the prepared sample.) Keep the tip immersed while the ampule fills completely.



4. Quickly invert the ampule several times to mix, then wipe off any liquid or fingerprints.

If barium is present, a white turbidity will develop.



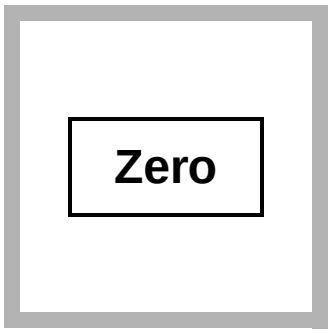
5. Touch the timer icon.

Touch **OK**.

A five-minute reaction period will begin.

Do not disturb the sample during the reaction period.

6. When the timer beeps, place the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0 mg/L Ba^{2+}

8. Within five minutes after the timer beep, place the prepared sample into the cell holder.

Results will appear in mg/L Ba^{2+}.

Interferences

Interfering Substance	Interference Levels and Treatments
Calcium	10,000 mg/L as $CaCO_3$
Magnesium	100,000 mg/L as $CaCO_3$
Silica	500 mg/L
Sodium Chloride	130,000 mg/L as NaCl
Strontium	Interferes at any level. If present, the total concentration between barium and strontium may be expressed as a PS (Precipitated by Sulfate). While this does not distinguish between barium and strontium, it gives an accurate indication of scaling tendency.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment (see *Section 3.3 Interferences* on page *50)*.

Sample Collection, Storage, and Preservation

Collect samples in an acid cleaned glass or plastic container. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter) (Cat. No. 2540-49). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 5 with 5.0 N sodium hydroxide (Cat. No. 2040-53). Correct the test result for volume additions (see *Section 3.1.3 Correcting for Volume Additions* on page *43*).

Standard Solutions

Prepare a 90.0-mg/L barium standard solution as follows:

1. Pipet 9.00 mL of Barium Standard Solution, 1000-mg/L, into a 100-mL volumetric flask.
2. Dilute to the mark with deionized water.
3. Prepare this solution daily. Perform the barium procedure as described above.

To adjust the calibration curve using the reading obtained with the 90.0-mg/L standard solution:

1. Touch **Options** on the current program menu. Touch **Standard Adjust** on the Options menu.
2. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *49* for more information.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the unspiked sample in the instrument. Verify that the units displayed are in mg/L.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Open a Barium Standard Solution, 1000-mg/L Ba.
5. Prepare a 0.1 mL sample spike by adding 0.1 mL of standard to the unspiked sample. Touch the timer icon. After the timer beeps, read the result.
6. Prepare a 0.2 mL sample spike by adding 0.1 mL of standard to the 0.1 mL sample spike. Touch the timer icon. After the timer beeps, read the result.
7. Prepare a 0.3 mL sample spike by adding 0.1 mL of standard to the 0.2 mL sample spike. Touch the timer icon. After the timer beeps, read the result. Each addition should reflect approximately 100% recovery.

Note: *For AccuVac Ampuls, fill three mixing cylinders (Cat. No. 1896-41) with 50 mL of sample and spike with 0.2 mL, 0.4 mL, and 0.6 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers. Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by touching* **Read***. Each addition should reflect approximately 100% recovery.*

8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *46* for more information.

Method Performance

Standard: 30 mg/L Ba

Program	95% Confidence Limits of Distribution
20	25–35 mg/L Ba
25	25–35 mg/L Ba

See *Section 3.4.3 Precision* on page *53* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
20	Entire Range	0.010	1 mg/L Ba
25	Entire Range	0.010	1 mg/L Ba

See *Section 3.4.5 Sensitivity* on page *54* for more information.

Calibration Standard Preparation

Prepare calibration standard containing 10, 20, 30, 50, 80, 90, and 100 mg/L Ba as follows:

1. Into seven different 100-mL Class A volumetric flasks, pipet 1, 2, 3, 5, 8, 9 and 10 mL of the 1000-mg/L Barium Standard Solution using Class A glassware.
2. Dilute to the mark with deionized water. Mix thoroughly.
3. Using the turbidimetric method and the calibration procedure described in the *User-Entered Programs* section of the instrument manual, generate a calibration curve from the standards prepared above.

Summary of Method

The BariVer® 4 Barium Reagent Powder combines with barium to form a barium sulfate precipitate, which is held in suspension by a protective colloid. The amount of turbidity present caused by the fine white dispersion of particles is directly proportional to the amount of barium present. Test results are measured at 450 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
BariVer® 4 Barium Reagent Powder Pillows	1 pillow	100/pkg	12064-99
or BariVer® 4 Barium Reagent AccuVac® Ampuls	1 ampul	25/pkg	25130-25

Required Apparatus

Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06
Beaker, 50-mL	1	each	500-41H

Required Standards

Barium Standard Solution, 1000-mg/L Ba		100 mL	14611-42
Water, deionized		4 liters	272-56

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Cadmium

Method 8017 — Dithizone Method[1]

Powder Pillows — (0 to 80.0 µg/L)

Scope and Application: For water and wastewater; digestion is required to determine total cadmium.

[1] Adapted from Standard Methods for the Examination of Water and Wastewater.

Test Preparation

Before starting the test:

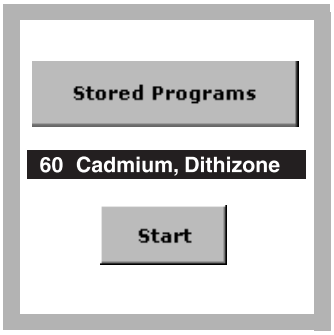
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample.
Clean all glassware with 6 N Hydrochloric Acid Solution and rinse with deionized water.
Cloudy and turbid samples may require filtering before running the test. Report results as µg/L soluble cadmium. Use glass membrane type filter to avoid loss of cadmium by adsorption onto the filter paper.
If samples cannot be analyzed immediately, see Sample Collection, Preservation, and Storage on page 4. Adjust the pH of preserved samples before analysis.
The Flow Cell and Sipper Modules cannot be used with this procedure.
The DithiVer powder will not completely dissolve in the chloroform. For further notes see Dithiver Solution Preparation and Storage on page 5.
Read the MSDS before testing. Spilled reagent will affect test accuracy and is hazardous to skin and other materials.

Collect the following items:	**Quantity**
Citrate buffer powder pillows	1
Chloroform	30 mL
DithiVer Metals Reagent powder pillows	1
Potassium Cyanide	0.1 g
Sodium Hydroxide solution, 50%	20 mL
Cotton balls	1
Clippers	1
Cylinder, 25-mL graduated	1
Cylinder, 250-mL graduated	1
Cylinder, 50-mL graduated mixing	1
Funnel, 500-mL separatory	1
Sample Cells	2
Spoon, measuring, 0.1-g	1
Support ring (4-inch) and stand (5 x 8-inch base)	1

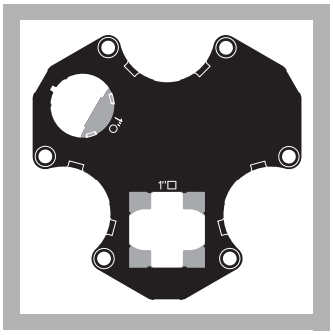
Note: *Reorder information for consumables and replacement items is on page 7.*

Powder Pillows — Method 8017

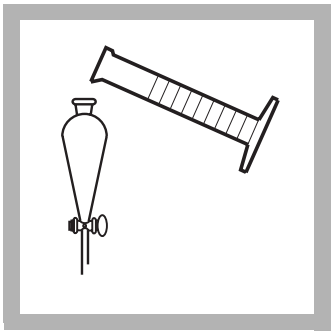
DANGER
Cyanide is a deadly poison. Use a fume hood. Maintain cyanide solutions at pH 11 or greater to prevent formation of cyanide gas.



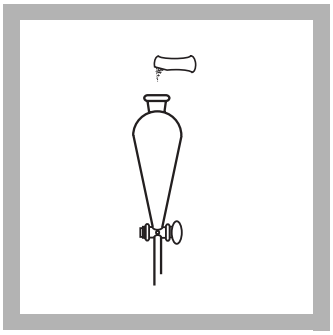
1. Select the test.



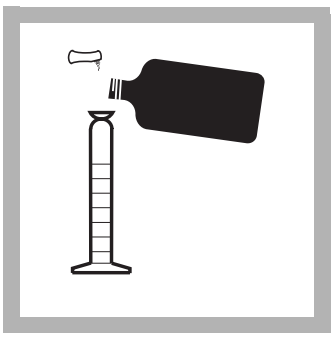
2. Insert the Multi-cell Adapter with the 1-inch square cell holder facing the user.



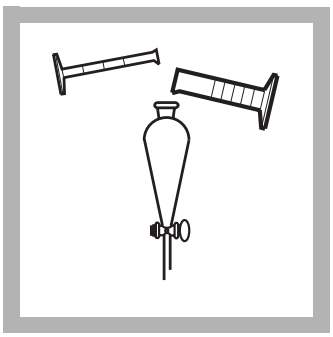
3. Fill a 250-mL graduated cylinder to the 250-mL mark with sample. Pour the sample into a 500-mL separatory funnel.



4. Add the contents of one Buffer Powder Pillow for heavy metals, citrate type. Stopper the funnel and shake to dissolve.



5. DithiVer Solution Preparation:
Add 50 mL of chloroform to a 50-mL mixing graduated cylinder. Add the contents of one DithiVer Metals Reagent Powder Pillow. Stopper the cylinder. Invert several times to mix.

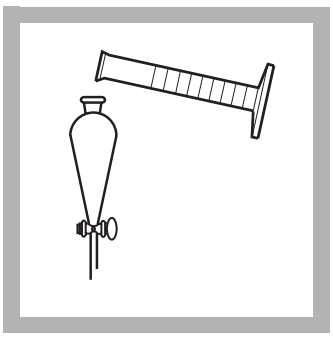


6. Add 20 mL of 50% Sodium Hydroxide Solution. Add a 0.1-g scoop of potassium cyanide to the funnel. Stopper. Shake vigorously for 15 seconds.

7. Remove the stopper.

Press **TIMER>OK**.

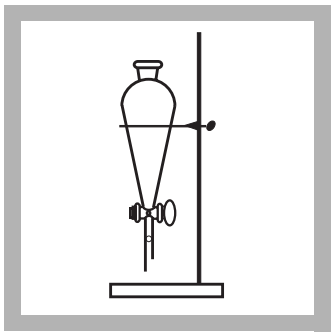
A 1-minute reaction period will begin.



8. Add 30 mL of the DithiVer solution to the 500-mL separatory funnel. Stopper, invert, and open stopcock to vent. Close the stopcock and shake funnel once or twice; vent again.

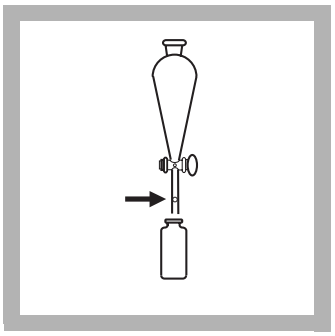
9. Press **TIMER>OK**.

Close the stopcock and shake the funnel vigorously during the 1 minute time period.



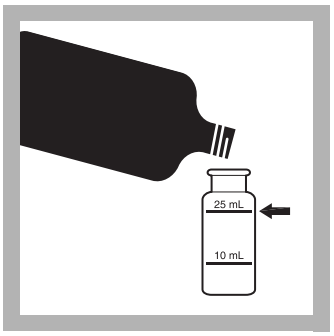
10. Press **TIMER>OK** and allow the funnel to stand undisturbed until the timer beeps.

The bottom (chloroform) layer will be orange to pink if cadmium is present.



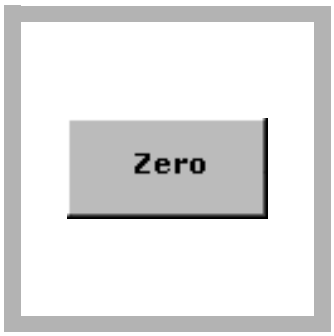
11. Prepared Sample: Insert a cotton plug the size of a pea into the delivery tube of the funnel and slowly drain the bottom (chloroform) layer into a dry 25-mL sample cell (the prepared sample). Stopper.

The cadmium-dithizone complex is stable for more than one hour if the sample cell is kept tightly capped and out of direct sunlight.



12. Blank Preparation: Fill a dry 25-mL sample cell with chloroform. Stopper.

13. Insert the blank into the cell holder with the fill line facing the user. Close the light shield.



14. Press **ZERO**.

The display will show:

0.0 µg/L Cd

15. Insert the prepared sample into the cell holder with the fill line facing the user. Close the light shield. Results are in µg/L cadmium.

Interferences

Table 1 Interfering Substances and Levels

Interfering Substance	Interference Levels and Treatments
Highly buffered samples or Extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.
Bismuth	Greater than 80 mg/L. See treatment below.
Copper	Greater than 2 mg/L. See treatment below.
Mercury	All levels. See treatment below.
Silver	Greater than 2 mg/L. See treatment below.

Table 2 Substances That Do Not Interfere

Aluminum	Lead
Antimony	Magnesium
Arsenic	Manganese
Calcium	Nickel
Chromium	Tin
Cobalt	Zinc
Iron	

To eliminate interference from the metals in Table 1, insert the following steps into the procedure after step 5.

1. Measure about 5-mL of the DithiVer solution into the separatory funnel. Stopper the funnel, invert and open the stopcock to vent. Close the stopcock and shake the solution vigorously for 15 seconds. Allow the funnel to stand undisturbed until the layers separate (about 30 seconds). A yellow, red, or bronze color in the bottom (chloroform) layer confirms the presence of interfering metals. Draw off and collect the bottom (chloroform) layer for proper disposal.

2. Repeat extraction with fresh 5 mL portions of the DithiVer solution (discarding the bottom layer each time) until the bottom layer shows a pure dark green color for three successive extracts. Extractions can be repeated several times without appreciably affecting the amount of cadmium in the sample.

3. Extract the solution with several 2- or 3-mL portions of pure chloroform to remove any remaining DithiVer, collecting the bottom layer each time for proper disposal.

4. Continue with Step 6 of the procedure.

5. In Step 8, substitute 28.5 mL of DithiVer solution for the 30 mL.

6. Continue with Step 9 of the procedure.

Sample Collection, Preservation, and Storage

Collect samples in an acid-washed glass or plastic containers. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Store preserved samples up to six months at room temperature. Adjust the pH to 2.5 with 5.0 N sodium hydroxide before analysis. Correct the test result for volume additions.

Dithiver Solution Preparation and Storage

Store DithiVer Powder Pillows away from light and heat. A convenient way to prepare this solution is to add the contents of 16 DithiVer Metals Reagent Powder Pillows to a 500-mL bottle of chloroform and invert several times until well mixed (carrier powder may not dissolve completely). Store dithizone solution in an amber glass bottle. This solution is stable for 24 hours.

Accuracy Check

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.

2. Press **OPTIONS>MORE**. Press **STANDARD ADDITIONS**. A summary of the standard additions procedure will appear.

3. Press **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Press **EDIT** to change these values. After values are accepted, the unspiked sample reading will appear in top row. See the user manual for more information.

4. Snap the neck off a Cadmium Voluette Ampule Standard, 25-mg/L Cd (25,000-µg/L Cd).

5. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively to three 250-mL samples and mix each thoroughly.

6. Analyze each standard addition sample as described above. Accept the standard additions reading by pressing **READ**. Each addition should reflect approximately 100% recovery.

7. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for the matrix interferences. Press **IDEAL LINE** to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

Standard Solution Method

1. Prepare a 5.0-mg/L cadmium standard solution by pipetting 5.00 mL of Cadmium Standard Solution, 100-mg/L Cd, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily.

2. Pipet 2.00 mL of the 5.0-mg/L Cadmium Standard Solution into 248 mL of deionized water in a 500-mL separatory funnel. This is a 40-µg/L cadmium solution. Perform the cadmium test on this solution beginning with Step 4 of the procedure.

3. To adjust the calibration curve using the reading obtained with the standard solution, press **OPTIONS>MORE** on the current program menu. Press **STANDARD ADJUST**.

4. Press **ON**. Press **ADJUST** to accept the displayed concentration. If an alternate concentration is used, press the number in the box to enter the actual concentration, then press **OK**. Press **ADJUST**.

Method Performance

Precision
Standard: 40.0 µg/L Cd

Program	95% Confidence Limits of Distribution
60	39.3–40.7 µg/L Cd

Sensitivity

Portion of Curve:	ΔAbs	ΔConcentration
Entire Range	0.010	0.73 µg/L

Summary of Method

The dithizone method is designed for the determination of cadmium in water and wastewater. The DithiVer Metals Reagent is a stable powder form of dithizone. Cadmium ions in basic solution react with dithizone to form a pink to red cadmium-dithizonate complex, which is extracted with chloroform. Test results are measured at 515 nm.

Pollution Prevention and Waste Management

Both chloroform (D022) and cyanide (D003) solutions are regulated as hazardous wastes by the Federal RCRA. Do not pour these solutions down the drain. Chloroform solutions and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal with laboratory solvent waste. Collect the cyanide solution as a reactive waste. Be sure that cyanide solutions are stored in a caustic solution with a pH >11 to prevent potential release of hydrogen cyanide gas. See the current MSDS for disposal information.

Consumables and Replacement Items

Required Reagents

Description	Quantity/Test	Unit	Cat. No.
Cadmium Reagent Set (100 Tests)	—	—	22422-00
Includes:(1) 14202-99, (1) 14458-17, (1) 12616-99, (1) 767-14, (4) 2180-49, (1) 2572-01			
Buffer Powder Pillows, citrate	1	100/pkg	14202-99
Chloroform, ACS	30 mL	4 L	14458-17
DithiVer Metals Reagent Powder Pillows	1	100/pkg	12616-99
Potassium Cyanide	0.1 g	125 g	767-14
Sodium Hydroxide Solution, 50%	20 mL	500 mL	2180-49
Cotton Balls, absorbent	1	100/pkg	2572-01

Required Apparatus

Description	Quantity/Test	Unit	Cat. No.
Clippers, for opening powder pillows	1	each	968-00
Cylinder, graduated, 25-mL	1	each	508-40
Cylinder, graduated, 250-mL	1	each	508-46
Cylinder, graduated, mixing, 50-mL	1	each	1896-41
Sample Cell, 1-in. Square, 25 mL with cap	2	2/pkg	26126-02
Funnel, separatory, 500-mL	1	each	520-49
Spoon, measuring, 0.1-g	1	each	511-00
Support Ring, 4"	1	each	580-01
Support Ring Stand, 5" x 8" base	1	each	563-00

Recommended Reagents and Standards

Description	Unit	Cat. No.
Cadmium Standard Solution, 100-mg/L Cd	100 mL	14024-42
Cadmium Standard Solution, 10-mL Voluette Ampule, 25-mg/L Cd	16/pkg	14261-10
Chloroform, ACS	500 mL	14458-49
Hydrochloric Acid Solution, 6.0 N	500 mL	884-49
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	2450-32
Sodium Hydroxide Standard Solution, 5.0 N	59 mL SCDB	2450-26
Water, deionized	4 L	272-56

Recommended Apparatus

Description	Unit	Cat. No.
Ampule Breaker Kit	each	21968-00
Cylinder, graduated, 5-mL	each	508-37
Filter Discs, glass, 47 mm	100/pkg	2530-00
Filter Holder, glass, for 47-mm filter	each	2340-00
Flask, Erlenmeyer, 500-mL	each	505-49
Flask, filtering, 500-mL	each	546-49
Flask, volumetric, Class A, 100-mL	each	14574-42
Flask, volumetric, Class A, 250-mL	each	14574-46
Flask, volumetric, Class A, 1000-mL, with glass stopper	each	14574-53
Hot Plate, 3½-in. diameter, 120 VAC, 50/60 Hz	each	12067-01
Hot Plate, 3½-in. diameter, 240 VAC, 50/60 Hz, variable control	each	12067-02
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
pH Meter, *sens**ion***™***1,** portable	each	51700-00
Pipet Filler, safety bulb	each	14651-00
Pipet, serological, 2-mL	each	532-36
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for TenSette Pipet 19700-01	50/pkg	21856-96
Pipet, volumetric, 2.00-mL, Class A	each	14515-36
Pipet, volumetric, 3.00-mL, Class A	each	14515-03
Pipet, volumetric, 6.00-mL, Class A	each	14515-06
Pipet, volumetric, 8.00-mL, Class A	each	14515-08
Pipet, volumetric, 9.00-mL, Class A	each	14515-09
Pipet, volumetric, 10.00-mL, Class A	each	14515-38
Pipet, volumetric, 20.00-mL, Class A	each	14515-20
Tongs, crucible, 9-inch	each	569-00

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Updated April 05 Edition 1

Chloride

Method 8113

Mercuric Thiocyanate Method
(0.1 to 25.0 mg/L Cl^-)

Scope and Application: For water and wastewater

Test Preparation

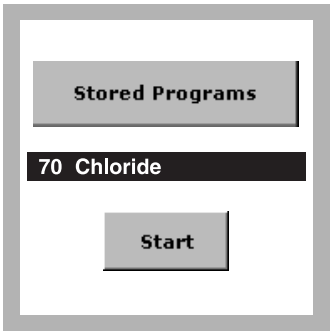
Before starting the test:

Filter turbid samples with moderately rapid filter paper and a funnel before analysis.
Both the sample and the blank will contain mercury (D009) at a concentration regulated as a hazardous waste by the Federal RCRA. Do not pour these solutions down the drain. Refer to the MSDS for more information on proper disposal of these materials.

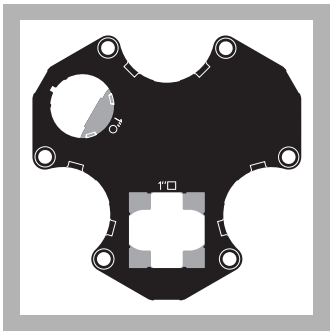
Collect the following items:	**Quantity**
Ferric Ion Solution	1 mL
Mercuric Thiocyanate Solution	2 mL
Deionized Water	10 mL
Sample Cells, 1-inch square, 10-mL	2
Pipet, TenSette®, 0.1 to 1.0 mL	1
Pipet tips for 0.1 to 1.0 mL TenSette pipet	2

Note: *Reorder information for consumables and replacement items is on page 4.*

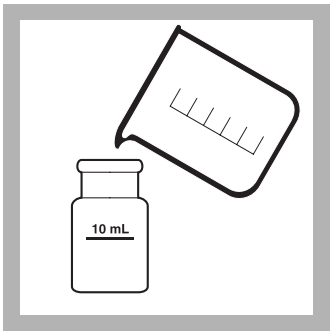
Mercuric Thiocyanate — Method 8113



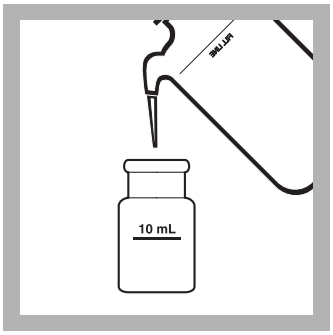
1. Select the test.



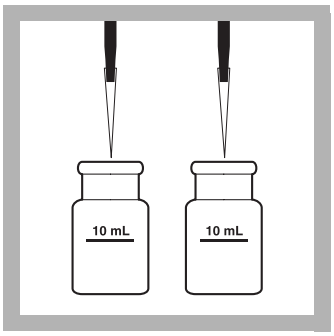
2. Install the Multi-cell Adapter with the 1-inch square cell position facing the user



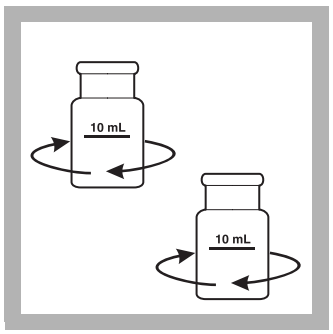
3. Prepared Sample: Fill a square sample cell with 10 mL of sample.



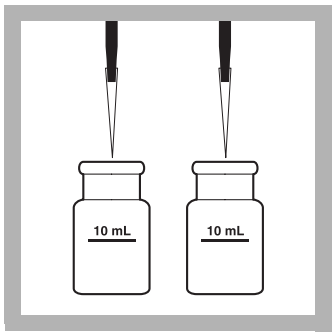
4. Blank Preparation: Fill another square sample cell with 10 mL of deionized water.



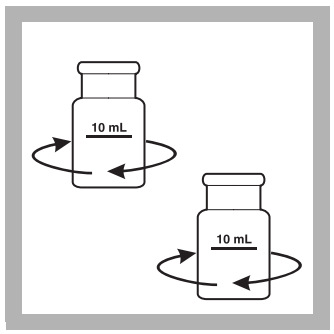
5. Pipet 1.0 mL of Mercuric Thiocyanate Solution into each sample cell.



6. Swirl to mix.



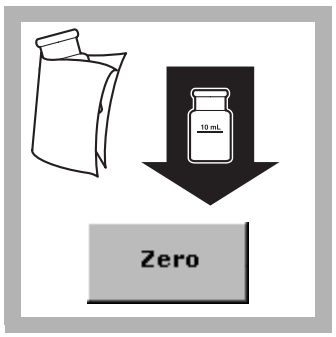
7. Pipet 0.5 mL of Ferric Ion Solution into each sample cell.



8. Swirl to mix. An orange color will develop if chloride is present.

9. Press **TIMER>OK**.

A two-minute reaction time will begin.



10. Within five minutes after the timer expires, wipe the blank and insert it into the cell holder with the fill line facing the user.

Press **ZERO**.

The display will show:

0.0 mg/L Cl^-

11. Wipe the prepared sample and insert it into the cell holder with the fill line facing the user.

Results are in mg/L Cl^-.

Interferences

Table 1 Interfering Substances and Levels

Interfering Substance	Interference Levels and Treatments
Extreme pH	Should be about pH 2 after adding reagents. If the sample is strongly acidic or alkaline, adjust a portion of sample before testing to a pH of about 7. Use either 5.0 N Sodium Hydroxide Standard Solution[1] or a 1:5 dilution of perchloric acid. **Use pH paper**; most pH electrodes will contaminate the sample with chloride.

[1] See Optional Reagents and Apparatus on page 4.

Sample Collection, Storage, and Preservation

Collect samples in glass or plastic containers. Samples can be stored for at least 28 days at room temperature.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Press **OPTIONS>MORE**. Press **STANDARD ADDITIONS**. A summary of the standard additions procedure will appear.
3. Press **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Press **EDIT** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Prepare three sample spikes. Fill three 50 mL mixing cylinders with 50 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of 1000-mg/L Chloride Standard Solution, respectively, to the cylinders and mix each thoroughly.
5. Analyze a 10 mL portion of each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by pressing **READ**. Each addition should reflect approximately 100% recovery.
6. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Press **IDEAL LINE** to view relationships between the sample spikes and the “Ideal Line” of 100% recovery.

Standard Solution Method

Prepare a 20.0-mg/L chloride standard solution as follows:

1. Using Class A glassware, pipet 10.00 mL of Chloride Standard Solution*, 1000-mg/L, into a 500-mL volumetric flask.
2. Dilute to the mark with deionized water. Perform the chloride procedure as described above.
3. To adjust the calibration curve using the reading obtained with the standard solution, press **OPTIONS>MORE** on the current program menu. Press **STANDARD ADJUST**.
4. Press **ON**. Press **ADJUST** to accept the displayed concentration. If an alternate concentration is used, press the number in the box to enter the actual concentration, then press **OK**. Press **ADJUST**.

Method Performance

Precision

Standard: 20.0 mg/L Cl^-

Program	95% Confidence Limits
70	17.9–22.1 mg/L Cl^-

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
1.0 mg/L	0.010	0.1 mg/L Cl^-
10.0 mg/L	0.010	0.3 mg/L Cl^-
20.0 mg/L	0.010	0.6 mg/L Cl^-

*See Optional Reagents and Apparatus on page 4.

Summary of Method

Chloride in the sample reacts with mercuric thiocyanate to form mercuric chloride and liberate thiocyanate ion. Thiocyanate ions react with the ferric ions to form an orange ferric thiocyanate complex. The amount of this complex is proportional to the chloride concentration. Test results are measured at 455 nm.

Consumables and Replacement Items

Required Reagents

Description	Quantity/Test	Unit	Cat. No.
Chloride Reagent Set (50 Tests)[1], includes:	—	each	23198-00
(1) Ferric Ion Solution	1 mL	100 mL	22122-42
(1) Mercuric Thiocyanate Solution	2 mL	200 mL	22121-29
Water, deionized	10 mL	4 L	272-56

[1] 50 tests equals 25 samples and 25 blanks.

Required Apparatus

Description	Quantity/Test	Unit	Cat. No.
Sample Cells, 1-inch square, 10-mL	2	2/pkg	24954-02
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for TenSette Pipet 19700-01	varies	50/pkg	21856-96

Recommended Standards

Description	Unit	Cat. No.
Chloride Standard Solution, 1000-mg/L Cl^-	500 mL	183-49

Optional Reagents and Apparatus

Description	Unit	Cat. No.
Chloride Standard Solution, 2-mL Voluette® Ampule, 12,500-mg/L Cl^-	20/pkg	14250-20
Cylinders, mixing	50 mL	1896-41
Filter Paper, funnel	100/pkg	692-57
Funnel, poly	75 mm	1083-68
Perchloric Acid, ACS	—	757-65
pH Paper	—	391-33
Pipet Tips, for TenSette Pipet 19700-01	1000/pkg	21856-28
Pipet, volumetric, Class A	1 mL	14515-35
Pipet, volumetric, Class A	0.5 mL	14515-37
Pipet Filler, safety bulb	—	14651-00
Sodium Hydroxide Standard Solution, 50 mL SCDB	50 mL	2450-26

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Updated April 05 Edition 1

Chromium, Total

Method 8024 — Alkaline Hypobromite Oxidation Method[1, 2]

Powder Pillows — (0.01 to 0.70 mg/L)

Scope and Application: For water and wastewater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater*

[2] This procedure is equivalent to Standard Method 3500-CRD for wastewater.

Test Preparation

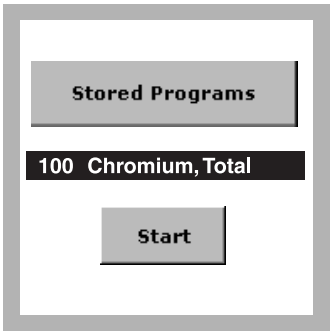
Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
Prepare a boiling water bath for step 5. Use finger cots to handle hot sample cells.

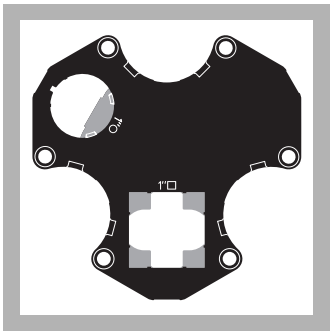
Collect the following items:	Quantity
Acid Reagent Powder Pillows	1
ChromaVer® 3 Chromium Reagent Powder Pillows	1
Chromium 1 Reagent Powder Pillows	1
Chromium 2 Reagent Powder Pillows	1
Hot Plate	1
Water Bath and Rack	1
Finger Cots	varies
Sample Cells, 1-inch square	2
Sample Cell, 1-inch round, 10–20–25 mL, with cap	1

Note: *Reorder information for consumables and replacement items is on page 5.*

Powder Pillows — Method 8024

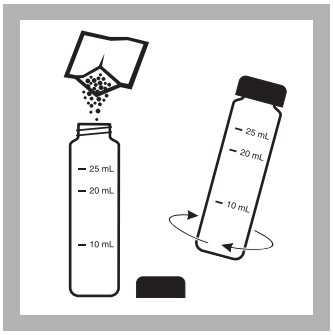


1. Select the test.

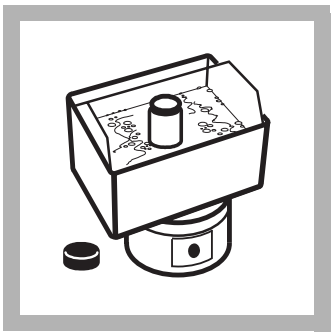


2. Insert the Multi-cell Adapter with the 1-inch square cell holder facing the user.

3. Fill a 25-mL sample cell with 25 mL of sample.



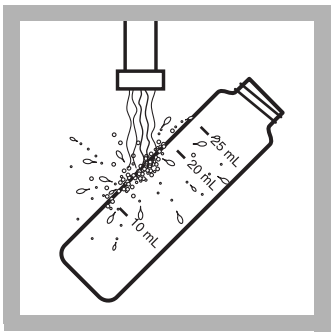
4. Prepared Sample: Add the contents of one Chromium 1 Reagent Powder Pillow. Swirl to mix.



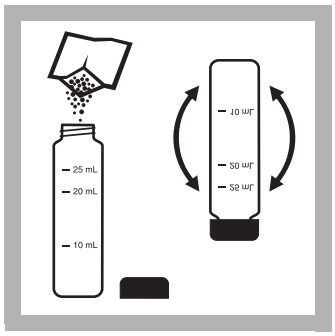
5. Insert the prepared sample into a boiling water bath.

6. Press **TIMER>OK**.

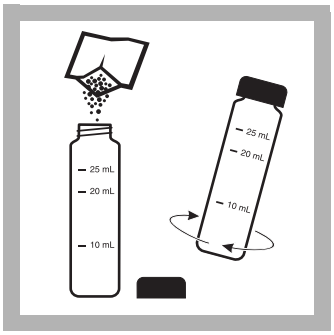
A five-minute reaction period will begin.



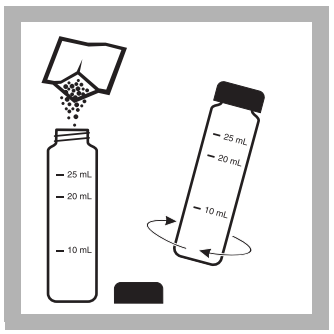
7. When the timer expires, remove the prepared sample. Using running water, cool the cell to 25 °C.



8. Remove cap and add the contents of one Chromium 2 Reagent Powder Pillow. Cap and invert to mix.



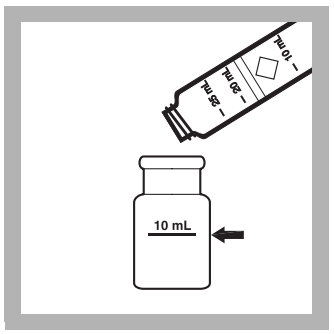
9. Add the contents of one Acid Reagent Powder Pillow. Swirl to mix.



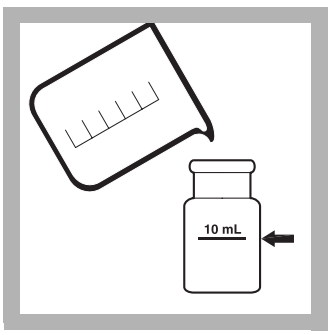
10. Add the contents of one ChromaVer 3 Chromium Reagent Powder Pillow. Swirl to mix.

11. Press **TIMER>OK**.

A five-minute reaction period will begin.

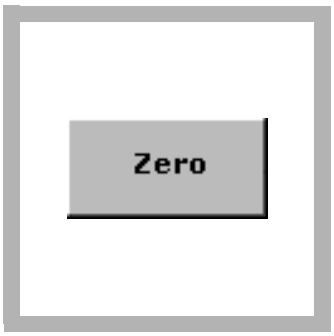


12. While the sample is reacting, pour 10 mL from the mixing bottle into a square sample cell.



13. Blank Preparation: When the timer expires, fill another sample cell with 10 mL of sample.

14. Wipe the blank and insert it into the cell holder with the fill line facing the user.



15. Press **Zero**.

The display will show:

0.00 mg/L Cr

16. Wipe the prepared sample and insert it into the cell holder with the fill line facing the user.

Results are in mg/L Cr.

Interferences

Table 1 Interfering Substances and Levels

Interfering Substance	Interference Levels and Treatments
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.
Organic material	May inhibit complete oxidation of trivalent chromium. If high levels of organic material are present, digestion may be required. Perform the analysis as described in this procedure on the digested sample.
Turbidity	For turbid samples, treat the 25-mL blank and the sample the same during steps 3–9.

Sample Collection, Storage, and Preservation

Collect samples in acid-washed glass or plastic containers. To preserve samples, adjust the pH to 2 or less with nitric acid. This requires approximately 2 mL per liter of the acid. Store preserved samples at room temperature up to six months. Adjust the pH to about 4 with 5.0 N Sodium Hydroxide before analysis. Correct the test result for volume additions.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.

2. Press **OPTIONS>MORE**. Press **STANDARD ADDITIONS**. A summary of the standard additions procedure will appear.

3. Press **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Press **EDIT** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.

4. Snap the neck off a Trivalent Chromium Voluette® Ampule Standard, 12.5-mg/L as Cr^{3+}.

5. Prepare three sample spikes. Fill three mixing cylinders with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.

6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by pressing **READ**. Each addition should reflect approximately 100% recovery.

7. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Press **IDEAL LINE** to view relationships between the sample spikes and the “Ideal Line” of 100% recovery.

Standard Solution Method
Prepare a 0.50-mg/L trivalent chromium standard as follows:

1. Dilute 5.00 mL of Trivalent Chromium Standard Solution, 50-mg/L as Cr^{3+}, to 500 mL with deionized water. Prepare this solution daily.

2. To adjust the calibration curve using the reading obtained with the standard solution, press **OPTIONS>MORE** on the current program menu. Press **STANDARD ADJUST**.

3. Press **ON**. Press **ADJUST** to accept the displayed concentration. If an alternate concentration is used, press the number in the box to enter the actual concentration, then press **OK**. Press **ADJUST**.

Method Performance

Precision
Standard: 0.50 mg/L Cr

Program	95% Confidence Limits of Distribution
100	0.47–0.53 mg/L Cr

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.005 mg/L Cr

Summary of Method

Trivalent chromium in the sample is oxidized to the hexavalent form by hypobromite ion under alkaline conditions. The sample is acidified. The total chromium content is determined by the 1,5-Diphenylcarbohydrazide method. Determine trivalent chromium by subtracting the results of a separate hexavalent chromium test from the results of the total chromium test.
Test results are measured at 540 nm.

Consumables and Replacement Items

Required Reagents

Description	Quantity/Test	Unit	Cat. No.
Total Chromium Reagent Set (100 tests), includes:	—	—	22425-00
Acid Reagent Powder Pillows	1	100/pkg	2126-99
ChromaVer® 3 Chromium Reagent Powder Pillows	1	100/pkg	12066-99
Chromium 1 Reagent Powder Pillows	1	100/pkg	2043-99
Chromium 2 Reagent Powder Pillows	1	100/pkg	2044-99

Required Apparatus

Description	Quantity/Test	Unit	Cat. No.
Sample Cells, 1-inch square, 10 mL, matched pair	2	2/pkg	24954-02
Sample Cell, 10-20-25 mL, with cap	1	each	24019-06
Hot plate, 3½-inch diameter, 120 VAC, 50/60 Hz	1	each	12067-01
OR			
Hot Plate, 4-inch diameter, 240 VAC, 50/60 Hz	1	each	12067-02
Water Bath and Rack	1	each	1955-55

Recommended Standards

Description	Unit	Cat. No.
Chromium, Trivalent, Standard Solution, 50-mg/L Cr^{3+}	100 mL	14151-42
Chromium, Trivalent, Standard Sol., 12.5-mg/L Cr^{3+}, Voluette Ampule®, 10-mL	16/pkg	14257-10

Optional Reagents and Apparatus

Description	Cat. No.
Acid Reagent Powder Pillow	2126-99
Finger Cots	14647-02
Flask, volumetric, Class A, 500-mL	14574-49
PIpette, volumetric, Class A, 5.00 mL	14515-37

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Updated April 05 Edition 1

Low Range Color, True and Apparent

Method 8025

Platinum-Cobalt Standard Method[1, 2]

LR (3 to 200 units)

Scope and Application: For water, wastewater, and seawater; equivalent to NCASI method 253 for pulp and paper effluent using 465 nm (requires pH adjustment)

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater* and *NCASI, Technical Bulletin No. 253*, Dec. 1971

[2] Adapted from *Wat. Res.* Vol. 30, No. 11, pp. 2771–2775, 1996

Test Preparation

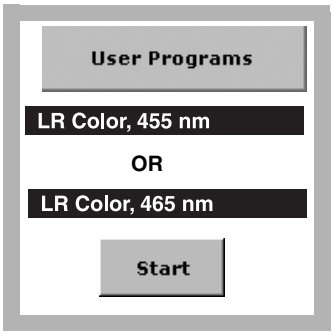
Before starting the test:

NCASI procedure requires pH adjustment. Adjust the pH to 7.6 with 1.0 N HCl or 1.0 N NaOH. When adjusting the pH, if overall volume change is greater than 1%, start over and use a stronger acid or base. Use the 465 nm wavelength setting when performing the NCASI procedure.
A minimum of 6 mL of sample is required for use with the 5-cm cell.
See Method Technique on page 4 for precautions in doing low level color measurements.
To test for **apparent color**, omit steps 4–6 and step 10. Use unfiltered deionized water in step 7 and unfiltered sample in step 11.

Collect the following items:	**Quantity**
Hydrochloric Acid Solution, 1.0 N (NCASI method at 465 nm only)	varies
Sodium Hydroxide, 1.00 N (NCASI method at 465 nm only)	varies
Water, deionized	100 mL
FIlter Apparatus: membrane filter, filter holder, filter flask, and aspirator	1
Sample Cell, 5-cm, glass or quartz	1
Stopper, rubber, one hole, No. 7	1
Tubing, rubber	1

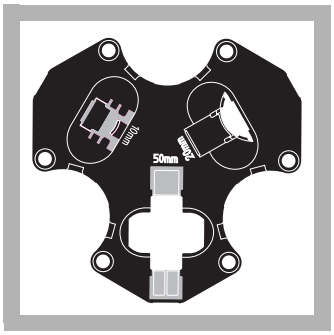
Note: *Reorder information for consumables and replacement items is on page 7.*

Platinum-Cobalt Method 8025

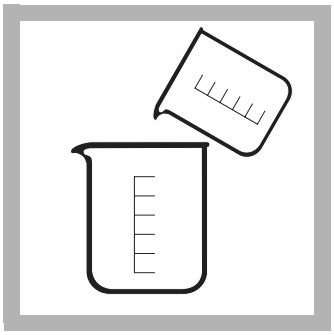


1. Select the test.

See Instrument Setup on page 5 for programming instructions.

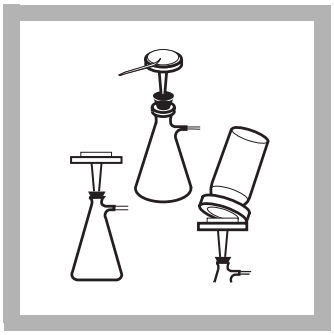


2. Insert the Multi-cell Adapter with the 50-mm rectangular cell holder facing the user.



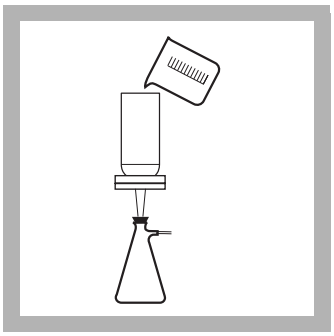
3. Collect 200 mL of sample in a 400-mL beaker.

NCASI: Adjust the pH as described in Test Preparation.

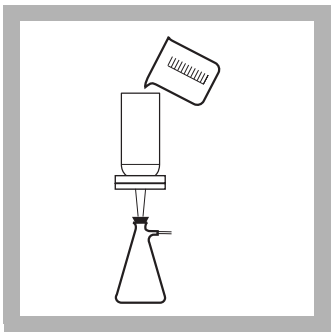


4. Assemble the filtering apparatus (0.45 micron membrane filter, filter holder, filter flask, and aspirator).

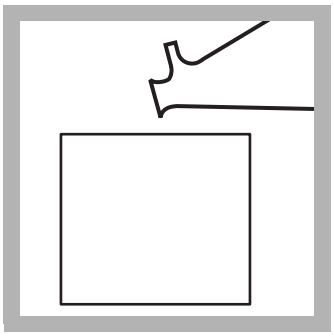
NCASI: Test prescribes a 0.8-micron filter.



5. Rinse the filter by pouring about 50 mL of deionized water through the filter. Discard the rinse water.

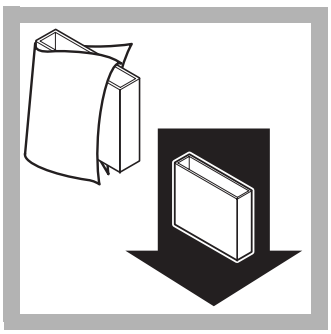


6. Pour another 50 mL of deionized water through the filter.



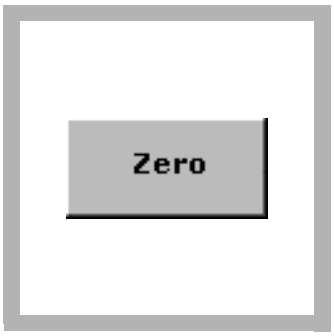
7. Blank Preparation: Fill a 5-cm (50-mm) pathlength cell with 10 mL of filtered deionized water from step 6.

Discard the excess water in the flask. Drain the flask well to avoid diluting the sample.



8. Carefully wipe the blank and check the cell for air bubbles or smudges on the cell windows.

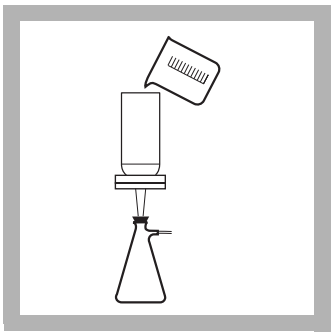
Insert the cell into the cell holder and close the lid.



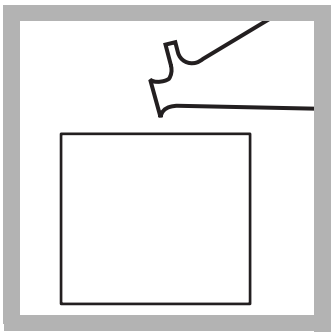
9. Press **ZERO**.

The display will show:

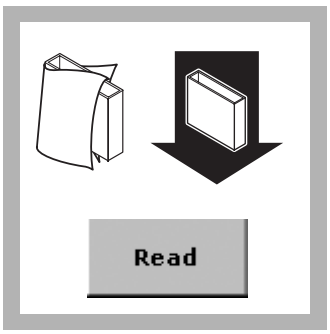
0 units PtCo_50_mm



10. Pour about 50 mL of sample through the filter.



11. Prepared Sample: Remove the Blank from the cell holder. Discard the deionized water. Rinse the cell twice with the filtered sample and then fill with 10 mL of sample.



12. Wipe the prepared sample and check for air bubbles or smudges on the cell windows.

Insert the cell into the cell holder. Close the lid.

Press **READ**.

Results are in PtCo color units.

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, fill bottles completely and cap tightly. Avoid excessive agitation or prolonged contact with air. Samples can be stored for 24 hours by cooling to 4 °C (39 °F). Warm to room temperature before analysis.

Accuracy Check

Standard Solution Method

Use a 15 Platinum-Cobalt Color Standard to fill a 5-cm cell. Carefully wipe the cell, check for air bubbles, and use in place of sample in Step 12.

Alternately, a 15 mg/L Pt-Co standard can be prepared by pipetting 15.0 mL of a 500 Pt-Co Color Standard into a 500 mL volumetric flask and diluting to volume with deionized water.

Method Performance

Precision
Standard: 15 units Pt-Co

Program	95% Confidence Limits of Distribution
455 nm	14–16 units Pt-Co
465 nm	14–16 units Pt-Co

Sensitivity

Program	Portion of Curve	∆Abs	∆Concentration
455 nm	Entire Range	0.010	7.4 units Pt-Co
465 nm	Entire Range	0.010	7.6 units Pt-Co

Method Technique

Zeroing and Reading the Sample
It is important to use extremely good lab technique and attention to detail in order to obtain reproducible results on waters which have true color values of less than 15 Pt-Co color units.

1. Wash the 5-cm sample cell with soap and water, or acid-rinse the cell to remove any traces of dirt, grease, finger prints, etc. Rinse several times with filtered deionized water.

2. Fill the cleaned cell with at least 6 mL of deionized water. Carefully wipe the cell windows with a lint-free towel.

3. View the cell through the cell windows to check for trapped air bubbles or traces of lint or smudges on or in the cell.

4. Tap the cell lightly on a bench or table to dislodge any trapped air bubbles.

5. Reproducibly place the cell into the cell holder and ‘zero’ the instrument. Insert the cell completely into the holder to properly seat the cell. The cell may initially fit tightly in the cell adapter, but will loosen slightly with additional use. There may be slight variability between cell manufacturers.

6. Remove the cell and repeat the above procedure for cleaning the cell.

7. Fill the cell again with deionized water and place in the cell holder and read the value. The instrument should read “0” if the sample cell is properly cleaned, no bubbles are present, and the cell is reproducibly placed in the cell holder.

Filtering the Sample
It is questionable if apparent color values are meaningful in low level color measurements. Any turbidity or suspended particles will contribute to the measured color value and will cause high results. It is therefore recommended that all samples for low level color measurements be first filtered through a membrane filter and the results be reported as true color. After the instrument has been zeroed, discard the deionized water and rinse the cell at least two times with the filtered sample. Fill the sample with filtered sample, check for bubbles or smudges and place the cell into the holder to make the measurement.

Cleaning the Sample Cells

Thoroughly rinse the sample cell with deionized water and dry after making sample measurements. Place the cell in a dust-free environment and dedicate the cell for LR color measurements only. The sample cleaning procedure will only be needed to be repeated as needed in future sample measurements.

Summary of Method

Color may be expressed as “apparent” or “true” color. The apparent color includes that from dissolved materials plus that from suspended matter. By filtering the suspended materials, the true color can be determined. The procedure describes true color analysis. If apparent color is desired, it can be determined by measuring an unfiltered water sample. The user program is used for both forms of color.

The user program is calibrated in color units based on the APHA-recommended standard of 1 color unit being equal to 1 mg/L platinum as chloroplatinate ion. Test results are measured at 455 and 465 nm, respectively.

Instrument Setup

Program Setup for 455 nm Method

1. Turn the DR 5000 ON and allow the instrument to complete the DR 5000 Self-Check

2. Follow the Display prompts and Enter commands for the 455 nm method (Table 1).

Table 1 LR Color 455 nm Program Setup

Step	Display Shows	Enter	Select
1	Main Menu	User Programs	—
2	User Programs	Program Options	—
3	Program Options	New	—
4	Program Number (950-999)?	Select a Program Number	**OK**
5	Program Name?	LR_Color_455_nm	**NEXT**
6	Program Type	Single Wavelength	**NEXT**
7	Units	Units	**NEXT**
8	Wavelength (nm)	455	**NEXT**
9	Concentration Resolution	1	**NEXT**
10	Chemical Form?	PtCo_50_mm	**NEXT**
11	Calibration	Enter Formula	**NEXT**
12	Enter Formula	b=737.85	**OK>DONE**
13	User Program for number assigned	Upper Limit	**EDIT**
14	Upper Limit	ON>220	**OK>OK**
15	User Program for number assigned	Lower Limit	**EDIT**
16	Lower Limit	ON>3	**OK>OK>STORE**

3. Exit and select the Program Number assigned for LR Color at 455 nm from the User Programs Menu to run the test.

Program Setup for 465nm—NCASI Method

1. Turn the DR 5000 ON and allow the instrument to complete the DR 5000 Self-Check.

2. Follow the Display prompts and Enter commands for the 465 nm method (Table 2).

Table 2 LR Color 465 nm NCASI Program Setup

Step	Display Shows	Enter	Select
1	Main Menu	User Programs	—
2	User Programs	Program Options	—
3	Program Options	New	—
4	Program Number (950-999)?	Select a Program Number	**OK**
5	Program Name?	LR_Color_465_nm	**NEXT**
6	Program Type	Single Wavelength	**NEXT**
7	Units	Units	**NEXT**
8	Wavelength (nm)	465	**NEXT**
9	Concentration Resolution	1	**NEXT**
10	Chemical Form?	PtCo_50_mm	**NEXT**
11	Calibration	Enter Formula	**NEXT**
12	Enter Formula	b=764.15	**OK>DONE**
13	User Program for number assigned	Upper Limit	**EDIT**
14	Upper Limit	ON>220	**OK>OK**
15	User Program for number assigned	Lower Limit	**EDIT**
16	Lower Limit	ON>3	**OK>OK>STORE**

3. Exit and select the Program Number assigned for LR Color at 465 nm from the User Programs Menu.

Consumables and Replacement Items

Required Reagents

Description	Quantity/Test	Unit	Cat. No.
Hydrochloric Acid Solution, 1.0 N (NCASI method at 465 nm only)	varies	1 L	23213-53
Sodium Hydroxide, 1.00 N (NCASI method at 465 nm only)	varies	900 mL	1045-53
Water, deionized	100 mL	4 L	272-56

Required Apparatus

Description	Quantity/Test	Unit	Cat. No.
Aspirator, Nalgene vacuum pump	1	each	2131-00
Filter Holder, magnetic, 47-mm	1	each	13529-00
Filter, membrane, 47-mm, 0.8-microns (for NCASI procedure)	1	100/pkg	26408-00
Filter, membrane, 47-mm, 0.45-microns	1	100/pkg	28947-00
Flask, filtering, 500-mL	1	each	546-49
Sample Cell, 5 cm (50 mm) pathlength, rectangular glass	1	each	26292-50
Stopper, rubber, one hole, No. 7	1	6/pkg	2119-07
Tubing, rubber, 2.4 mm wall	1	12 ft	560-19

Recommended Standards and Apparatus

Description	Unit	Cat. No.
Color Standard Solution, 500 platinum-cobalt units	1L	1414-53
Color Standard Solution, 15 platinum-cobalt units	1 L	26028-53

Optional Standards and Apparatus

Description	Unit	Cat. No.
Detergent, Liqui-Nox	1 L	20881-53
Flask, volumetric, Class A, 500 mL	each	14574-49
Hydrochloric Acid; 6.0 N, 1:1	500 mL	884-49
Pipet, volumetric, Class A, 15.00 mL	each	14515-39
Pipet Filler, safety bulb	each	14651-00
Sample Cell, 5 cm (50 mm) pathlength, rectangular quartz,	each	26244-50
Wipers, Disposable	280/pk	20970-00

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Updated December 2005 Edition 1

Copper

★Method 8506 and Method 8026

Powder Pillows or AccuVac® Ampuls

Bicinchoninate Method[1]

(0.04 to 5.00 mg/L)

Scope and Application: For water, wastewater and seawater[2]; Method 8506 USEPA approved for reporting wastewater analysis (digestion required)[3]

[1] Adapted from Nakano, S., *Yakugaku Zasshi*, 82 486-491 (1962) [*Chemical Abstracts,* 58 3390e (1963)]

[2] Pretreatment required; see *Interferences (Using Powder Pillows)*

[3] *Federal Register*, 45 (105) 36166 (May 29, 1980)

Test Preparation

Before starting the test:

Digestion is required for determining total copper.
Adjust the pH of acid-preserved samples to 4–6 with 8 N KOH before analysis.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

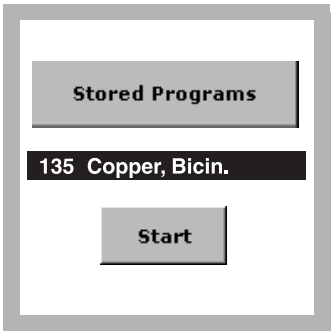
Collect the following items:	**Quantity**
Powder Pillow Test:	
CuVer® 1 Copper Reagent powder pillow	1
Sample Cells, 1-in. square, 10-mL (Powder Pillow Test)	2
AccuVac Test:	
CuVer® 2 Copper Reagent AccuVac® Ampul	1
Beaker, 50-mL (AccuVac test)	1
Sample Cell, 10-mL (AccuVac test)	1

Note: *Reorder information for consumables and replacement items is on page 6.*

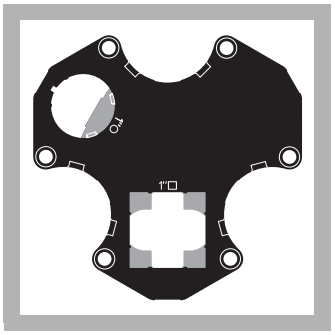
Note: *If copper is present, the sample will turn purple when it mixes with the reagent powder.*

Note: *Accuracy is not affected by undissolved powder.*

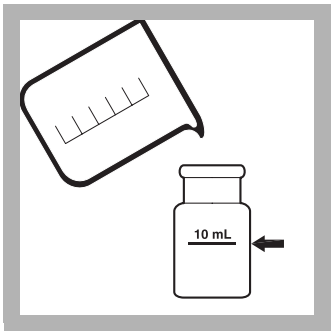
Powder Pillows — Method 8506



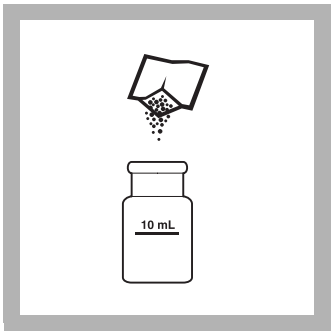
1. Select the test.



2. Install the Multi-cell Adapter with the 1-inch square cell holder facing the user.



3. **Prepared Sample:** Fill a square sample cell with 10 mL of sample.

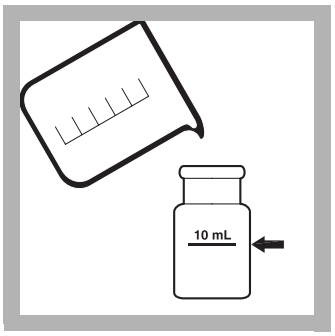


4. Add the contents of one CuVer® 1 Copper Reagent Powder Pillow to the sample cell (the prepared sample). Twirl sample cell to mix.

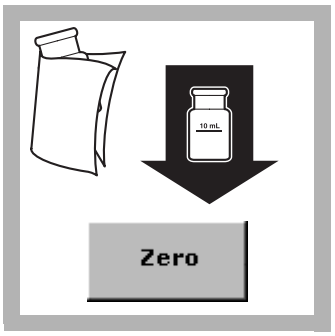
Use a CuVer 2 Copper Reagent Pillow for samples containing high levels of aluminum, iron, and hardness. A 25-mL sample cell is required. See Table 1.

5. Press **TIMER>OK**.

A 2-minute reaction period will begin.



6. **Blank Preparation:** When the timer expires, fill a second square sample cell with 10 mL of sample.



7. Insert the blank into the cell holder with the fill line facing the user.

Press **ZERO**.

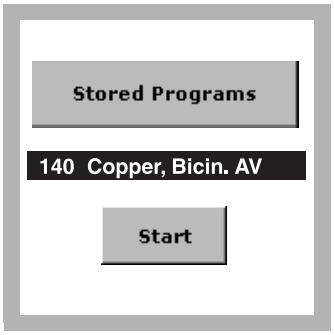
The display will show:

0.00 mg/L Cu

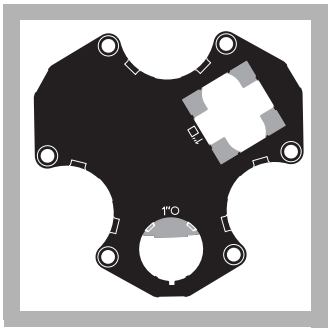
8. Within 30 minutes after the timer expires, insert the prepared sample into the cell holder with the fill line facing the user.

Results are in mg/L Cu.

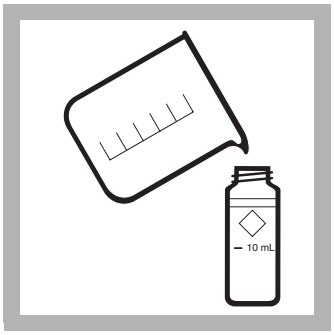
AccuVac® Ampul Method 8026



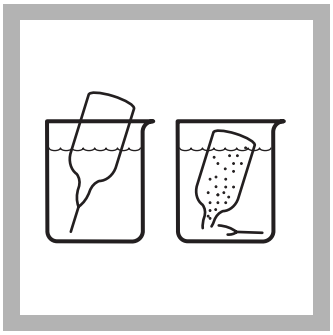
1. Select the test.



2. Install the Multi-cell Adapter with the round cell holder facing the user.

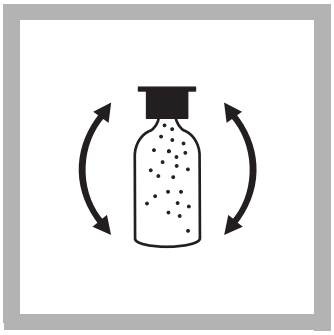


3. Blank Preparation: Fill a round sample cell with 10-mL of sample.



4. Prepared Sample: Collect at least 40 mL of sample in a 50-mL beaker.

Fill a CuVer 2 AccuVac Ampul with sample. Keep the tip immersed while the ampule fills completely.



5. Quickly invert the ampule several times to mix. Wipe off any liquid or fingerprints with cloth or soft paper towel.

6. Press **TIMER>OK**

A 2-minute reaction period will begin.

7. When the timer expires, insert the blank into the cell holder. Close the cover.

Press **ZERO**.

The display will show:

0.00 mg/L Cu

8. Within 30 minutes after the timer expires, insert the AccuVac Ampul into the cell holder.

Results are in mg/L Cu.

Interferences

To differentiate free copper from that complexed to EDTA or other complexing agents, use a 25-mL sample cell and Free Copper Reagent Powder Pillow instead of the CuVer 1 Powder Pillow in step 3. Results in step 8 will be free copper only. Add a Hydrosulfite Reagent Powder Pillow to the same sample and re-read the result. This result will include the total dissolved copper (free and complexed). Unlike CuVer 1 Reagent, CuVer 2 Reagent Powder Pillows and AccuVac Ampuls react directly with copper, which is complexed by chelants such as EDTA.

Table 1 Interfering Substances and Suggested Treatments for Powder Pillows

Interfering Substance	Interference Levels and Treatments
Acidity	If the sample is extremely acidic (pH 2 or less) a precipitate may form. Add 8 N Potassium Hydroxide Standard Solution drop-wise until sample pH is above 4. Continue with step 3.
Aluminum, Al^{3+}	Follow the powder pillow procedure above, but substitute a CuVer 2 Copper Reagent Powder Pillow for the CuVer 1 Pillow used in step 4. Results obtained will include total dissolved copper (free and complexed). Requires a 25-mL sample volume.
Cyanide, CN^-	Prevents full color development. Before adding the CuVer 1 Powder Pillow Reagent, add 0.2 mL of formaldehyde to the 10-mL sample. Wait 4 minutes before taking the reading. Multiply the test results by 1.02 to correct for sample dilution by the formaldehyde.
Hardness	Follow the powder pillow procedure above, but substitute a CuVer 2 Copper Reagent Powder Pillow for the CuVer 1 Pillow used in step 4. Results obtained will include total dissolved copper (free and complexed). Requires a 25-mL sample volume.
Iron, Fe^{3+}	Follow the powder pillow procedure above, but substitute a CuVer 2 Copper Reagent Powder Pillow for the CuVer 1 Pillow used in step 4. Results obtained will include total dissolved copper (free and complexed). Requires a 25-mL sample volume.
Silver, Ag^+	If a turbidity remains and turns black, silver interference is likely. Add 10 drops of saturated Potassium Chloride Solution to 75 mL of sample, followed by filtering through a fine or highly retentive filter. Use the filtered sample in the procedure.

Table 2 Interfering Substances and Suggested Treatments for AccuVac® Ampuls

Interfering Substance	Interference Levels and Treatments
Acidity	If the sample is extremely acidic (pH 2 or less) a precipitate may form. Add 8 N Potassium Hydroxide Standard Solution drop-wise until sample pH is above 4. Continue with step 3.
Aluminum, Al^{3+}	Reagents accommodate high levels.
Cyanide, CN^-	Prevents full color development. Add 0.5 mL of formaldehyde per 25-mL of sample before using CuVer 2 Reagent AccuVac Ampul. Wait 4 minutes before taking the reading. Multiply the test results by 1.02 to correct for sample dilution by the formaldehyde.
Hardness	Reagents accommodate high levels.
Iron, Fe^{3+}	Reagents accommodate high levels.
Silver, Ag^+	If a turbidity remains and turns black, silver interference is likely. Add 10 drops of saturated Potassium Chloride Solution to 75 mL of sample, followed by filtering through a fine or highly retentive filter. Use the filtered sample in the procedure.

Sample Collection, Storage, and Preservation

Collect samples in acid-cleaned glass or plastic containers. Adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Store preserved samples up to six months at room temperature. Before analysis, adjust the pH to 4–6 with 8 N Potassium Hydroxide. Do not exceed pH 6, as copper may precipitate. Correct the test result for volume additions. If only dissolved copper is to be determined, filter the sample before acid addition.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Press **OPTIONS>MORE**. Press **STANDARD ADDITIONS**. A summary of the standard additions procedure will appear.
3. Press **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Press **EDIT** to change these values. After values are accepted, the

unspiked sample reading will appear in the top row. See the user manual for more information.

4. Snap the neck off a Copper Voluette® Ampule Standard, 12.5-mg/L Cu.
5. Prepare a 0.1 mL sample spike by adding 0.1 mL of standard to the unspiked sample. Press the timer icon. After the timer beeps, read the result.
6. Prepare a 0.2 mL sample spike by adding 0.1 mL of standard to the 0.1 mL sample spike. Press the timer icon. After the timer beeps, read the result.
7. Prepare a 0.3 mL sample spike by adding 0.1 mL of standard to the 0.2 mL sample spike. Press the timer icon. After the timer beeps, read the result. Each addition should reflect approximately 100% recovery.

Note: *For AccuVac Ampuls, fill three mixing cylinders with 50-mL of sample and spike with 0.2 mL, 0.4 mL, and 0.6 mL of Copper Voluette Ampule Standard, 75-mg/L Cu. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers. Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by pressing* ***READ****. Each addition should reflect approximately 100% recovery.*

8. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Press **IDEAL LINE** to view relationships between the sample spikes and the “Ideal Line” of 100% recovery.

Standard Solutions Method
Prepare a 4.00-mg/L Standard as follows:

1. Using Class A glassware, pipet 4.00 mL of Copper Standard Solution, 100-mg/L as Cu, into a 100-mL volumetric flask. Dilute to volume with deionized water, stopper and invert to mix. Perform the procedure as described above.
2. To adjust the calibration curve using the reading obtained with the standard solution, press **OPTIONS>MORE** on the current program menu. Press **STANDARD ADJUST**.
3. Press **ON**. Press **ADJUST** to accept the displayed concentration. If an alternate concentration is used, press the number in the box to enter the actual concentration, then press **OK**. Press **ADJUST**.

Method Performance

Precision
Standard: 1.00 mg/L Cu

Program	95% Confidence Limits of Distribution
135	0.97–1.03 mg/L Cu
140	0.97–1.03 mg/L Cu

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
135	Entire range	0.010	0.04 mg/L Cu
140	Entire range	0.010	0.03 mg/L Cu

Summary of Method

Copper in the sample reacts with a salt of bicinchoninic acid contained in CuVer 1 or CuVer 2 Copper Reagent to form a purple colored complex in proportion to the copper concentration. Test results are measured at 560 nm.

Consumables and Replacement Items

Required Reagents

Description	Quantity/Test	Unit	Cat. No.
CuVer® 1 Copper Reagent Powder Pillows	1	100/pkg	21058-69
OR			
CuVer® 2 Copper Reagent AccuVac® Ampuls	1	25/pkg	25040-25

Required Apparatus (Powder Pillows)

Description	Quantity/Test	Unit	Cat. No.
Sample Cells, 1-inch square, 10 mL, matched pair	2	2/pkg	24954-02

Required Apparatus (AccuVac)

Description	Quantity/Test	Unit	Cat. No.
Beaker, 50-mL	1	each	500-41H
Sample Cell, 10-mL, with cap	1	6/pkg	24276-06

Recommended Standards

Description	Unit	Cat. No.
Copper Standard Solution, 100-mg/L as Cu	100 mL	128-42
Copper Voluette® Ampule Standard, 12.5-mg/L as Cu	16/pkg	21126-10
Copper Voluette® Ampule Standard, 75-mg/L as Cu, 2-mL	10/pkg	14247-10
Metals Drinking Water Standard, LR for Cu, Fe, Mn	500 mL	28337-49
Metals Drinking Water Standard, HR for Cu, Fe, Mn	500 ml/L	28336-49

Optional Reagents and Apparatus

Description	Cat. No.
Beakers, 50-mL	500-41H
CuVer 2 Copper Reagent Powder Pillow	21882-99
Cylinders, mixing	1896-41
Formaldehyde	2059-32
Nitric Acid, concentrated	152-49
Potassium Chloride Solution	765-42
Potassium Hydroxide Standard Solution, 8 N	282-32H
Reagent Set for Free and Total Copper, includes:	24392-00
Hydrosulfite Reagent Powder Pillows	21188-69
Free Copper Reagent Powder Pillows	21823-69
Sample Cells, 25 mL, with stoppers, 2/pkg	26126-02

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Updated April 05 Edition 1

Cyanide

Method 8027
Powder Pillows

Pyridine-Pyrazalone Method[1]
(0.002 to 0.240 mg/L CN^-)

Scope and Application: For water, wastewater, and seawater

[1] Adapted from Epstein, Joseph, *Anal. Chem.* 19(4), 272 (1947)

Test Preparation

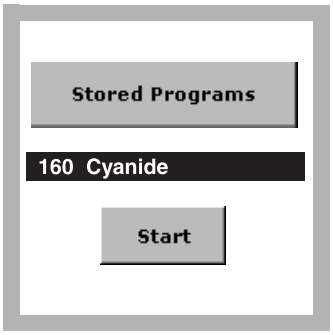
Before starting the test:

Use a water bath to maintain the optimum temperature for the reaction in this test (25 °C). Samples at less than 23 °C require longer reaction times, and samples at greater than 25 °C yield low results.
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
The timing for steps 3–10 is critical. You may find it useful to open the necessary reagents before starting this sequence.
All samples to be analyzed for cyanide should be treated by acid distillation except when experience has shown that there is no difference in results obtained with or without distillation. See Acid Distillation on page 5.
See Pollution Prevention and Waste Management on page 3 for proper disposal of solutions containing cyanide.

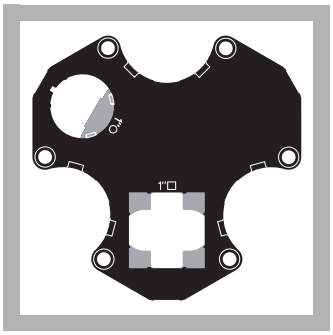
Collect the following items	Quantity
CyaniVer® Cyanide 3 Reagent Powder Pillow	1
CyaniVer® Cyanide 4 Reagent Powder Pillow	1
CyaniVer® Cyanide 5 Reagent Powder Pillow	1
Cylinder, graduated, 10-mL	1
Sample Cells, 1-inch square glass	2

Note: *Reorder information for consumables and replacement items is on page 7.*

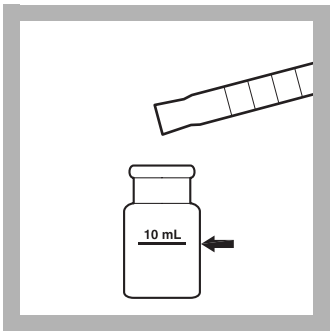
Powder Pillows — Method 8027



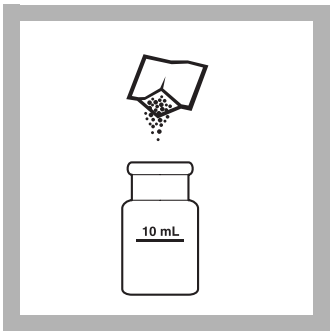
1. Select the test.



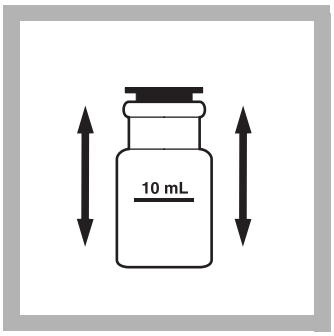
2. Insert the Multi-cell Adapter with the 1-inch square cell holder facing the user.



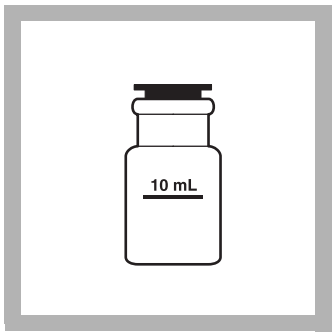
3. Using a graduated cylinder, fill a square sample cell with a 10 mL of sample.



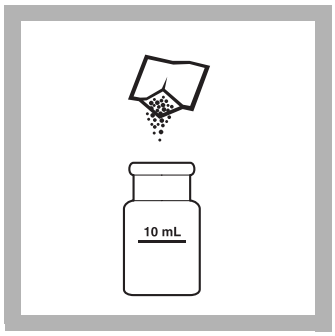
4. Prepared Sample: Add the contents of one CyaniVer 3 Cyanide Reagent Powder Pillow. Cap the cell.



5. Shake the sample cell for 30 seconds.

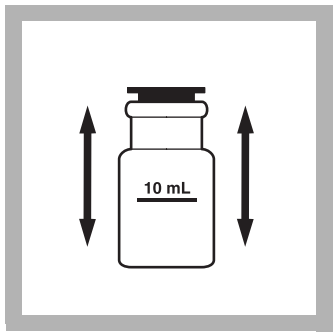


6. Leave the sample cell undisturbed for an additional 30 seconds.

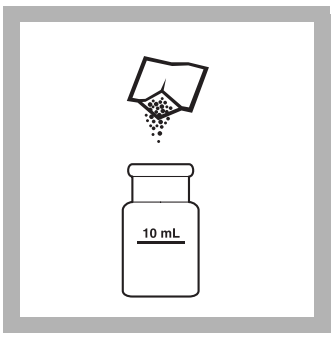


7. Add the contents of one CyaniVer 4 Cyanide Reagent Powder Pillow.

Cap the sample cell.

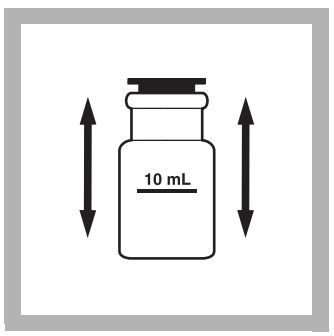


8. Shake the sample for 10 seconds. Immediately proceed to *step* 9. (Delaying the addition of the CyaniVer 5 will produce low test results.)



9. Add the contents of one CyaniVer 5 Cyanide Reagent Powder Pillow.

Cap the sample cell.



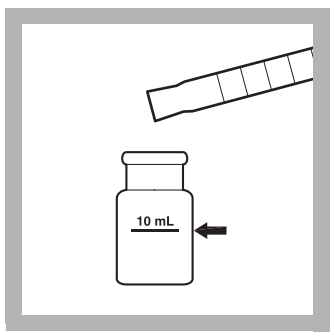
10. Shake the cell vigorously.

If cyanide is present, a pink color will develop.

11. Press **TIMER>OK**.

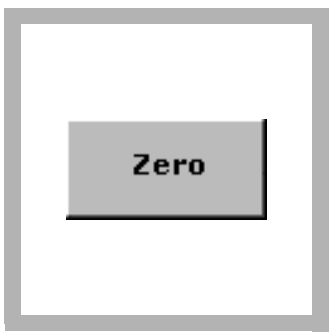
A 30-minute reaction period will begin.

The solution will turn from pink to blue.



12. Blank Preparation: When the timer expires, fill a second square sample cell with 10 mL of sample.

13. Wipe the blank and insert it into the cell holder with the fill line facing the user.



14. Press **ZERO**.

The display will show:

0.000 mg/L CN^-

15. Wipe the prepared sample and insert it into the cell holder with the fill line facing the user.

Results are in mg/L CN^-.

Pollution Prevention and Waste Management

Special Considerations for Cyanide Containing Materials
Samples analyzed by this procedure may contain cyanide, which is regulated as reactive (D003) waste by the federal RCRA. It is imperative these materials be handled safely to prevent the release of hydrogen cyanide gas (an extremely toxic material with the smell of almonds). Most cyanide compounds are stable and can be safely stored for disposal in highly alkaline solutions (pH >11) such as 2 N sodium hydroxide. Never mix these wastes with other laboratory wastes which may contain lower pH materials such as acids or even water.

In the event of a spill or release, special precautions must be taken to prevent exposure to hydrogen cyanide gas. The following steps may be taken to destroy the cyanide compounds in the event of an emergency:

- Use a fume hood or supplied air or self contained breathing apparatus.
- While stirring, add the waste to a beaker containing a strong solution of sodium hydroxide and calcium hypochlorite or sodium hypochlorite (household bleach).
- Maintain a strong excess of hydroxide and hypochlorite. Let the solution stand for 24 hours.
- Neutralize and flush the solution down the drain with a large excess of water.

Note: *If the solution contains other regulated materials such as chloroform or heavy metals, it may still need to be collected for hazardous waste disposal. Never flush hazardous wastes down the drain.*

Interferences

Table 1 Interfering Substances and Levels

Interfering Substance	Interference Levels and Treatments
Chlorine	Large amounts of chlorine in the sample will cause a milky white precipitate after the addition of the CyaniVer® 5 Reagent. If chlorine or other oxidizing agents are known to be present, pretreat the sample before testing using the procedure in this table for oxidizing agents.
Metals	Nickel or cobalt in concentrations up to 1 mg/L do not interfere. Eliminate the interference from up to 20 mg/L copper and 5 mg/L iron by adding the contents of one HexaVer Chelating Reagent Powder Pillow[1] to the sample and then mixing before adding the CyaniVer 3 Cyanide Reagent Powder Pillow in step 4. Prepare a reagent blank of deionized water and reagents to zero the instrument in step 14.
Oxidizing Agents	**1.** Adjust a 25-mL portion of the alkaline sample to pH 7–9 with 2.5 N Hydrochloric Acid Standard Solution[1]. Count the number of drops of acid added. **2.** Add two drops of Potassium Iodide Solution[1] and two drops of Starch Indicator Solution[1] to the sample. Swirl to mix. The sample will turn blue if oxidizing agents are present. **3.** Add Sodium Arsenite Solution[1] drop-wise until the sample turns colorless. Swirl the sample thoroughly after each drop. Count the number of drops. **4.** Take another 25-mL sample and add the total number of drops of Hydrochloric Acid Standard Solution counted in step a. **5.** Subtract one drop from the amount of Sodium Arsenite Solution added in step c. Add this amount to the sample and mix thoroughly. Continue with step 3 of the cyanide procedure.

Table 1 Interfering Substances and Levels (continued)

Interfering Substance	Interference Levels and Treatments
Reducing Agents	**1.** Adjust a 25-mL portion of the alkaline sample to pH 7–9 with 2.5 N Hydrochloric Acid Standard Solution[1]. Count the number of drops added. **2.** Add four drops of Potassium Iodide Solution[1] (Cat. No. 343-32) and four drops of Starch Indicator Solution to the sample. Swirl to mix. The sample should be colorless. **3.** Add Bromine Water[1] drop-wise until a blue color appears. Swirl the sample thoroughly after each addition. Count the number of drops. **4.** Take another 25-mL sample and add the total number of drops of Hydrochloric Acid Standard Solution counted in step a. **5.** Add the total number of drops of Bromine Water counted in *step c* to the sample and mix thoroughly. **6.** Continue with step 3 of the cyanide procedure.
Turbidity	Large amounts of turbidity will cause high readings. Use filter paper[1] and a funnel[1] to filter highly turbid water samples before use in steps 3 and 12. The test results should then be recorded as soluble cyanide.

[1] See Optional Reagents and Apparatus on page 8.

Sample Collection, Storage, and Preservation

Collect samples in glass or plastic bottles and analyze as quickly as possible.

The presence of oxidizing agents, sulfides and fatty acids can cause the loss of cyanide during sample storage. Samples containing these substances must be pretreated as described below before preservation with sodium hydroxide. If the sample contains sulfide and is not pretreated, it must be analyzed within 24 hours.

Preserve the sample by adding 4.0 mL of 5.0 N Sodium Hydroxide Standard Solution* to each liter (or quart) of sample, using a glass serological pipet and pipet filler. Check the sample pH; 4-mL of sodium hydroxide is usually enough to raise the pH of most water and wastewater samples to 12. Add more 5.0 N Sodium Hydroxide if necessary. Store the samples at 4 °C (39 °F) or less. Samples preserved in this manner can be stored for 14 days.

Before testing, samples preserved with 5.0 N Sodium Hydroxide or samples that are highly alkaline due to chlorination treatment processes or sample distillation procedures should be adjusted to approximately pH 7 with 2.5 N Hydrochloric Acid Standard Solution. Where significant amounts of preservative are used, a volume correction should be made.

Oxidizing Agents

Oxidizing agents such as chlorine decompose cyanides during storage. To test for their presence and to eliminate their effect, pretreat the sample as follows:

1. Take a 25-mL portion of the sample and add one drop of 10-g/L m-Nitrophenol Indicator Solution*. Swirl to mix.
2. Add 2.5 N Hydrochloric Acid Standard Solution drop-wise until the color changes from yellow to colorless. Swirl the sample thoroughly after the addition of each drop.
3. Add two drops of Potassium Iodide Solution*, 30-g/L, and two drops of Starch Indicator Solution, to the sample. Swirl to mix. The solution will turn blue if oxidizing agents are present.
4. If step 3 suggests the presence of oxidizing agents, add two level, 1-g measuring spoonfuls of Ascorbic Acid* per liter of sample.

* See Optional Reagents and Apparatus on page 8.

5. Withdraw a 25-mL portion of sample treated with ascorbic acid and repeat *steps 1* to *3*. If the sample turns blue, repeat *steps 4* and *5*.
6. If the 25-mL sample remains colorless, preserve the remaining sample to pH 12 for storage with 5 N Sodium Hydroxide Standard Solution* (usually 4-mg/L).
7. Perform the procedure given under Interfering Substances and Levels, Reducing Agents, to eliminate the effect of excess ascorbic acid, before following the cyanide procedure.

Sulfides
Sulfides will quickly convert cyanide to thiocyanate (SCN^-). To test for the presence of sulfide and eliminate its effect, pretreat the sample as follows:

1. Place a drop of sample on a disc of Hydrogen Sulfide Test Paper* that has been wetted with pH 4 Buffer Solution*.
2. If the test paper darkens, add a 1-g measuring spoon of Lead Acetate to the sample. Repeat *step a*.
3. If the test paper continues to turn dark, keep adding Lead Acetate* until the sample tests negative for sulfide.
4. Filter the lead sulfide precipitate through Filter Paper* and a Funnel*. Preserve the sample for storage with 5 N Sodium Hydroxide Standard Solution* or neutralize to a pH of 7 for analysis.

Fatty Acids
Caution
Perform this operation under a ventilation hood and as quickly as possible.

When distilled, fatty acids will pass over with cyanide and, under the alkaline conditions of the absorber, will form soaps. If the presence of fatty acid is suspected, use the following pretreatment before preserving samples with sodium hydroxide.

1. Acidify 500 mL of sample to pH 6 or 7 with a 4:1 dilution of glacial Acetic Acid*.
2. Pour the sample into a 1000-mL separation funnel and add 50 mL of Hexane*.
3. Stopper the funnel and shake for one minute. Allow the layers to separate.
4. Drain off the lower, sample layer into a 600-mL beaker. If the sample is to be stored, add enough 5 N Sodium Hydroxide Standard Solution* to raise the pH above 12.

Acid Distillation

All samples to be analyzed for cyanide should be treated by acid distillation except when experience has shown that there is no difference in results obtained with or without distillation. With most compounds, a one-hour reflux is adequate.

If thiocyanate is present in the original sample, a distillation step is absolutely necessary as thiocyanate causes a positive interference. High concentrations of thiocyanate can yield a substantial quantity of sulfide in the distillate. The “rotten egg” smell of hydrogen sulfide will accompany the distillate when sulfide is present. The sulfide must be removed from the distillate prior to testing.

If cyanide is not present, the amount of thiocyanate can be determined. The sample is not distilled and the final reading is multiplied by 2.2. The result is mg/L SCN^-.

The distillate can be tested and treated for sulfide after the last step of the distillation procedure by using the following lead acetate treatment procedure.

* See Optional Reagents and Apparatus on page 8.

1. Place a drop of the distillate (already diluted to 250 mL) on a disc of Hydrogen Sulfide Test Paper* that has been wetted with pH 4.0 Buffer Solution*.
2. If the test paper darkens, add 2.5 N Hydrochloric Acid Standard Solution* drop-wise to the distillate until a neutral pH is obtained.
3. Add a 1-g measuring spoon of lead acetate* to the distillate and mix. Repeat step 1.
4. If the test paper continues to turn dark, keep adding lead acetate until the distillate tests negative for sulfide.
5. Filter the black lead sulfide precipitate through filter paper* and a funnel*. Neutralize the liquid filtrate to pH 7 and immediately analyze for cyanide.

Distillation Procedure

The following steps describe the distillation process using distillation apparatus* and cyanide glassware* offered by the manufacturer:

1. Set up the distillation apparatus for cyanide recovery, leaving off the thistle tube. Refer to the *Distillation Apparatus Manual*. Turn on the water and make certain it is flowing steadily through the condenser.
2. Fill the distillation apparatus cylinder to the 50-mL mark with 0.25 N Sodium Hydroxide Standard Solution*.
3. Fill a clean 250-mL graduated cylinder to the 250-mL mark with sample and pour it into the distillation flask. Place a stirring bar into the flask and attach the thistle tube.
4. Arrange the vacuum system as shown in the Distillation Apparatus Manual, but do not connect the vacuum tubing to the gas bubbler. Turn on the water to the aspirator to full flow and adjust the flow meter to 0.5 SCFH.
5. Connect the vacuum tubing to the gas bubbler, making certain that air flow is maintained (check the flow meter) and that air is bubbling from the thistle tube and the gas bubbler.
6. Turn the power switch on and set the stir control to 5. Using a 50-mL graduated cylinder, pour 50 mL of 19.2 N Sulfuric Acid Standard Solution* through the thistle tube and into the distillation flask.
7. Using a water bottle, rinse the thistle tube with a small amount of deionized water.
8. Allow the solution to mix for three minutes; then add 20 mL Magnesium Chloride Reagent* through the thistle tube and rinse again. Allow the solution to mix for 3 more minutes.
9. Verify that there is a constant flow of water through the condenser.
10. Turn the heat control to 10.
11. Carefully monitor the distillation flask at this point in the procedure. Once the sample begins to boil, slowly lower the air flow to 0.3 SCFH. If the contents of the distillation flask begin to back up through the thistle tube, increase the air flow by adjusting the flow meter until the contents do not back up through the thistle tube. Boil the sample for one hour.
12. After one hour, turn off the still, but maintain the air flow for 15 minutes more.
13. After 15 minutes, remove the rubber stopper on the 500-mL vacuum flask to break the vacuum and turn off the water to the aspirator. Turn off the water to the condenser.
14. Remove the gas bubbler/cylinder assembly from the distillation apparatus. Separate the gas bubbler from the cylinder and pour the contents of the cylinder into a 250-mL, Class A volumetric flask. Rinse the gas bubbler, cylinder and J-tube connector with deionized water and add the washings to the volumetric flask.

* See Optional Reagents and Apparatus on page 8.

15. Fill the flask to the mark with deionized water and mix thoroughly. Neutralize the contents of the flask and analyze for cyanide.

Accuracy Check

Standard Solutions Method

CAUTION

Cyanides and their solutions, and the hydrogen cyanide liberated by acids, are very poisonous. Both the solutions and the gas can be absorbed through the skin.

Prepare a 100 mg/L cyanide stock solution weekly as follows:

1. Dissolve 0.2503 grams of potassium cyanide in deionized water and dilute to 1000 mL.
2. Immediately before use, prepare a 0.200 mg/L cyanide working solution by diluting 2.00 mL of the 100 mg/L stock solution to 1000 mL using deionized water.
3. To adjust the calibration curve using the reading obtained with the 0.200 mg/L standard solution, press **OPTIONS>MORE** on the current program menu. Press **STANDARD ADJUST**.
4. Press **ON**. Press **ADJUST** to accept the displayed concentration (the value depends on the selected units). If an alternate concentration is used, press the number in the box to enter the actual concentration, then press **OK**. Press **ADJUST**.

Method Performance

Precision

Standard: 0.100 mg/L CN^-

Program	95% Confidence Limits of Distribution
160	0.090–0.110 mg/L CN^-

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.002 mg/L CN^-

Summary of Method

The Pyridine-Pyrazalone method used for measuring cyanide gives an intense blue color with free cyanide. A sample distillation is required to determine cyanide from transition and heavy metal cyanide complexes. Test results are measured at 612 nm.

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Consumables and Replacement Items

Required Reagents

Description	Quantity/Test	Unit	Cat. No.
Cyanide Reagent Set, includes:	—	—	24302-00
(1) CyaniVer® 3 Cyanide Reagent Powder Pillow	1	100/pkg	21068-69
(1) CyaniVer® 4 Cyanide Reagent Powder Pillow	1	100/pkg	21069-69
(1) CyaniVer® 5 Cyanide Reagent Powder Pillow	1	100/pkg	21070-69

Required Apparatus

Description	Quantity/Test	Unit	Cat. No.
Cylinder, graduated, 10-mL	1	each	508-38
Sample Cells, 1-inch square, 10-mL, matched pair	2	2/pkg	24954-02
Stopper, poly, hollow	—	6/pkg	14480-00

Recommended Standards

Description	Unit	Cat. No.
Potassium Cyanide, ACS	125 g	767-14
Water, deionized	4 L	272-56

Optional Reagents and Apparatus

Description	Cat. No.
Acetic Acid	100-49
Ascorbic Acid	6138-26
Balance, analytical	28014-01
Bromine Water	2211-20
Buffer Solution, pH 4	12223-49
Filter Paper	1894-57
Funnel	1083-67
Hexane Solution	14478-49
HexaVer Chelating Reagent Powder Pillow	243-99
Hydrochloric Acid Standard Solution, 2.5 N	1418-32
Hydrogen Sulfide Test Paper	25377-33
m-Nitrophenol Indicator Solution, 10-g/L	2476-32
Magnesium Chloride Reagent	14762-53
Potassium Iodide Solution, 30-g/L	343-32
Sodium Arsenite Solution, 5-g/L	1047-32
Sodium Hydroxide Standard Solution, 0.25 N	14763-53
Sodium Hydroxide Standard Solution, 5.0 N	2450-53
Starch Indicator Solution	349-32
Sulfuric Acid Standard Solution, 19.2 N	2038-49
Cyanide Glassware	22658-00
Distillation Apparatus, 115 VAC	22744-00
Distillation Apparatus, 230 VAC	22744-02
Distillation Apparatus Set	22653-00

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HACH COMPANY
WORLD HEADQUARTERS
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FAX: (970) 669-2932

Updated April 05 Edition 1

Fluoride

★Method 8029 — SPADNS Method[1]

Reagent Solution or AccuVac® Ampuls — (0.02 to 2.00 mg/L F^-)

Scope and Application: For water, wastewater and seawater; USEPA accepted for reporting for drinking and wastewater analyses (distillation required; see *Distillation on page 4*)[2]

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater, 4500-F B & D*

[2] Procedure is equivalent to USEPA method 340.1 for drinking water and wastewater.

Test Preparation

Before starting the test:

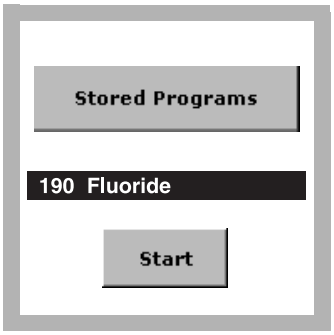
The sample and deionized water should be at the same temperature (±1 °C). Temperature adjustments may be made before or after reagent addition.
SPADNS Reagent is toxic and corrosive. Use care while handling the reagent.
For best results, measure the volume of SPADNS Reagent as accurately as possible.
If the instrument displays Over Measure Range!, dilute a fresh sample with an equal volume of deionized water and repeat the test, using this solution in step 3. Multiply the result by 2.

Collect the following items:	**Quantity**
Solution Test:	
SPADNS Reagent Solution	4 mL
Deionized Water	10 mL
Pipet, volumetric, 2-mL	1
Pipet, volumetric, 10-mL	1
Pipet Filler Bulb	1
Sample cells, 1-in. square, 10-mL	2
Thermometer, –10 to 110 °C	1
AccuVac Test:	
SPADNS Fluoride Reagent AccuVac® Ampuls	2
Deionized Water	40 mL
Beaker, 50-mL	1

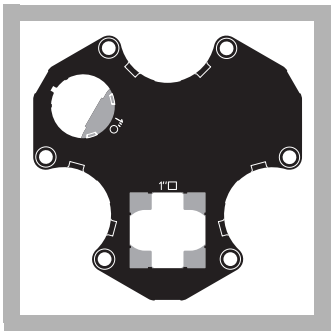
Note: *Reorder information for consumables and replacement items is on page 6.*

Important Note: *SPADNS Reagent contains sodium arsenite. Final solutions will contain arsenic (D004) in sufficient concentration to be regulated as a hazardous waste for Federal RCRA. Refer to the MSDS for disposal instructions.*

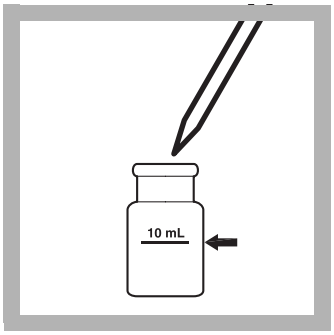
Using SPADNS Reagent Method 8029



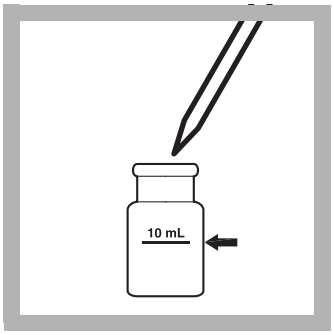
1. Select the test.



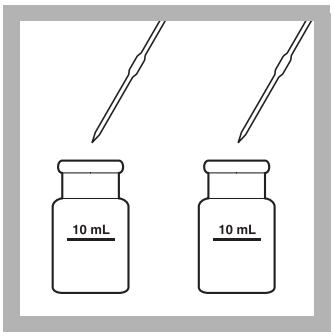
2. Install the Multi-cell Adapter with the 1-inch square cell holder facing the user.



3. Prepared Sample: Pipet 10.0 mL of sample into a dry square sample cell.



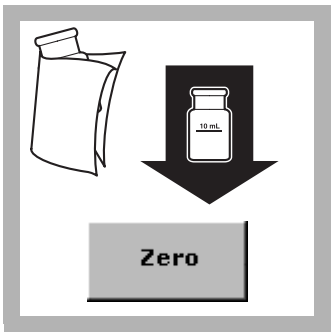
4. Blank Preparation: Pipet 10.0 mL of deionized water into a second dry square sample cell.



5. Carefully pipet 2.0 mL of SPADNS Reagent into each cell. Swirl to mix.

6. Press **TIMER>OK**.

A one-minute reaction period will begin.



7. When the timer expires, insert the blank into the cell holder with the fill line facing the user.

Press **ZERO**.

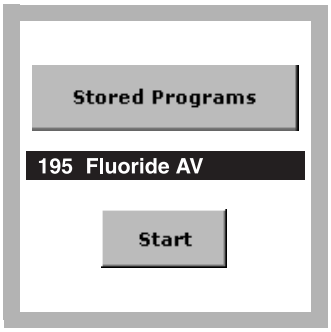
The display will show:

0.00 mg/L F⁻

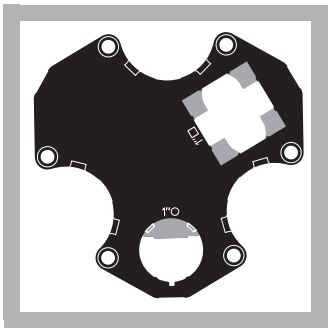
8. Insert the prepared sample into the cell holder with the fill line facing the user.

Results are in mg/L F⁻.

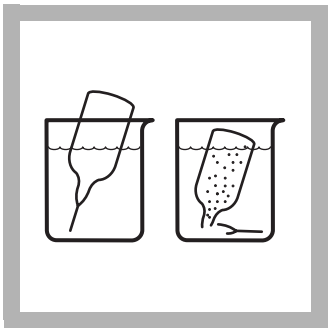
AccuVac® Ampul — Method 8029



1. Select the test.

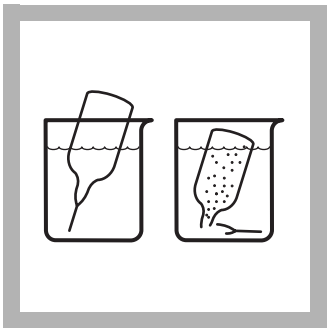


2. Install the Multi-cell Adapter with the round cell holder facing the user.



3. Prepared Sample: Collect at least 40 mL of sample in a 50-mL beaker.

Fill one SPADNS Fluoride Reagent AccuVac Ampul with sample. Keep the tip immersed while the ampule fills completely.



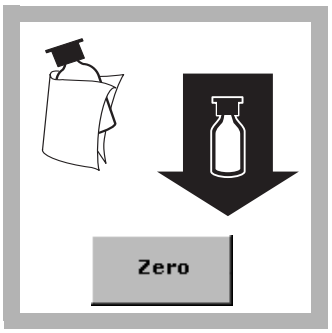
4. Blank Preparation: Pour at least 40 mL of deionized water into a second beaker.

Fill a second ampule with deionized water. Keep the tip immersed while the ampule fills completely.

5. Quickly invert both ampules several times to mix.

6. Press **TIMER>OK**.

A one-minute reaction period will begin.



7. When the timer expires, insert the blank into the cell holder. Press **ZERO**.

The display will show:

0.00 mg/L F⁻

8. Insert the AccuVac Ampul that contains the sample into the cell holder.

Results are in mg/L F⁻.

Interferences

This test is sensitive to small amounts of interference. Glassware must be very clean (acid rinse before each use). Repeat the test with the same glassware to ensure that results are accurate.

Table 1 Interfering Substances and Levels

Interfering Substance	Interference Levels and Treatments
Alkalinity (as $CaCO_3$)	At 5000 mg/L it causes a −0.1 mg/L F^- error.
Aluminum	At 0.1 mg/L it causes a −0.1 mg/L F^- error. To check for interferences from aluminum, read the concentration one minute after reagent addition, then again after 15 minutes. An appreciable increase in concentration suggests aluminum interference. Waiting 2 hours before making the final reading will eliminate the effect of up to 3.0 mg/L aluminum.
Chloride	At 7000 mg/L it causes a +0.1 mg/L F^- error.
Chlorine	SPADNS Reagent contains enough arsenite to eliminate interference up to 5 mg/L chlorine. For higher chlorine levels, add one drop of Sodium Arsenite Solution[1] to 25 mL of sample for each 2 mg/L of Chlorine.
Iron, ferric	At 10 mg/L it causes a −0.1 mg/L F^- error.
Phosphate, ortho	At 16 mg/L it causes a +0.1 mg/L F^- error.
Sodium Hexametaphosphate	At 1.0 mg/L it causes a +0.1 mg/L F^- error.
Sulfate	At 200 mg/L it causes a +0.1 mg/L F^- error.

[1] See Optional Reagents and Apparatus on page 7.

Distillation

Most interferences can be eliminated by distilling the sample from an acid solution as described below:

1. Set up the distillation apparatus for general purpose distillation. Refer to the Distillation Apparatus manual for proper assembly. Use a 125-mL Erlenmeyer flask to collect the distillate.
2. Turn on the water and maintain a steady flow through the condenser.
3. Measure 100 mL of sample into the distillation flask using a 100-mL graduated cylinder. Add a magnetic stir bar and 5 glass beads.
4. Turn the stirrer power switch on. Turn the stir control to 5.
5. Using a 250-mL graduated cylinder, carefully add 150 mL of StillVer® Distillation Solution into the flask. (StillVer Distillation Solution is a 2:1 mixture of concentrated sulfuric acid and water.)

Note: *When distilling samples with high amounts of chloride, add 5 mg of Silver Sulfate* to the sample for every mg/L of chloride in the sample.*

6. With the thermometer in place, turn the heat control to 10. The yellow pilot lamp indicates the heater is on.
7. When the temperature reaches 180 °C or when 100 mL of distillate has been collected, turn the still off (requires about 1 hour).
8. Dilute the distillate to a volume of 100 mL, if necessary. The distillate may now be analyzed by the SPADNS or the fluoride ion-selective electrode method.

Sample Collection, Storage, and Preservation

Samples may be stored in glass or plastic bottles for at least seven days when cooled to 4 °C (39 °F) or lower. Warm samples to room temperature before analysis.

* See Optional Reagents and Apparatus on page 7.

Accuracy Check

Standard Solution Method
A variety of standard solutions covering the entire range of the test is available. Use these instead of sample to verify technique.

Minor variations between lots of reagent become measurable above 1.5 mg/L. While results in this region are usable for most purposes, better accuracy may be obtained by diluting a fresh sample 1:1 with deionized water and retesting. Multiply the result by 2.

To adjust the calibration curve using the reading obtained with a standard solution:

1. Press **OPTIONS>MORE** on the current program menu. Press **STANDARD ADJUST>OFF**.
2. Press **ON**. Press **ADJUST** to accept the displayed concentration (the value depends on the selected units). If an alternate concentration is used, press the number in the box to enter the actual concentration, then press **OK**. Press **ADJUST**.

Method Performance

Precision
Standard: 1.00 mg/L F^-

Program	95% Confidence Limits of Distribution
190	0.97–1.03 mg/L F^-
195	0.92–1.08 mg/L F^-

Sensitivity

Sensitivity	ΔAbs	ΔConcentration (Program 190)	ΔConcentration (Program 195)
at 1 mg/L	0.010	0.024 mg/L F^-	0.03 mg/L F^-

Summary of Method

The SPADNS Method for fluoride determination involves the reaction of fluoride with a red zirconium-dye solution. The fluoride combines with part of the zirconium to form a colorless complex, thus bleaching the red color in an amount proportional to the fluoride concentration. This method is accepted by the EPA for NPDES and NPDWR reporting purposes when the samples have been distilled. Seawater and wastewater samples require distillation. Test results are measured at 580 nm.

Consumables and Replacement Items

Required Reagents

Description	Quantity/Test	Unit	Cat. No.
SPADNS Reagent Solution	4 mL	500 mL	444-49
OR			
SPADNS Fluoride Reagent AccuVac® Ampuls	2	25/pkg	25060-25
Water, deionized	10 mL	4 L	272-56

Required Apparatus (Solution)

Description	Quantity/Test	Unit	Cat. No.
Pipet Filler, safety bulb	1	each	14651-00
Pipet, volumetric, Class A, 2.00-mL	1	each	14515-36
Pipet, volumetric, Class A, 10.00-mL.	1	each	14515-38
Sample Cells, 1-inch square, 10 mL, matched pair	2	2/pkg	24954-02
Thermometer, –10 to 110 °C	1	each	1877-01

Required Apparatus (AccuVac)

Description	Quantity/Test	Unit	Cat. No.
Beaker, 50-mL	1	each	500-41H

Recommended Standards

Description	Unit	Cat. No.
Fluoride Standard Solution, 0.2-mg/L F^-	500 mL	405-02
Fluoride Standard Solution, 0.5-mg/L F^-	500 mL	405-05
Fluoride Standard Solution, 0.8-mg/L F^-	500 mL	405-08
Fluoride Standard Solution, 1.0-mg/L F^-	1000 mL	291-53
Fluoride Standard Solution, 1.0-mg/L F^-	500 mL	291-49
Fluoride Standard Solution, 1.2-mg/L F^-	500 mL	405-12
Fluoride Standard Solution, 1.5-mg/L F^-	500 mL	405-15
Fluoride Standard Solution, 2.0-mg/L F^-	500 mL	405-20
Fluoride Standard Solution, 100-mg/L F^-	500 mL	232-49
Standard, Drinking Water, Mixed Parameter, Inorganic for F^-, NO_3, PO_4, SO_4	500 mL	28330-49

Consumables and Replacement Items (continued)

Distillation Reagents and Apparatus

Description	Quantity/Test	Unit	Cat. No.
Cylinder, graduated, 100-mL	1	each	508-42
Cylinder, graduated, 250-mL	1	each	508-46
Distillation Heater and Support Apparatus Set,115 VAC, 50/60 Hz	1	each	22744-00
AND			
Distillation Heater and Support Apparatus Set,230 VAC, 50/60 Hz	1	each	22744-02
OR			
Distillation Apparatus Set, General Purpose	1	each	22653-00
Flask, Erlenmeyer, 125-mL	1	each	20897-43
Glass Beads	1	100/pkg	2596-00
StillVer® Distillation Solution	varies	500 mL	446-49
Stir Bar, magnetic	1	each	10764-16

Optional Reagents and Apparatus

Description	Cat. No.
Silver Sulfate	334-14
Sodium Arsenite Solution, 0.5 g/L	1047-32

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Updated April 05 Edition 1

Method 8317

LeadTrak®* Fast Column Extraction Method (5 to 150 µg/L)

Scope and Application: For drinking water

* Patent Number 5,019,516

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- The sampling requirements for "first-draw" analysis are detailed in *Sample Collection, Storage and Preservation on page 524*.
- Reagents will stain the sample cells, rinse the cells with 1:1 HNO_3, followed by deionized water.

Fast Column Extraction Method 8317

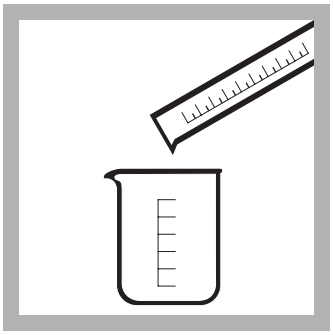
1. Touch

Hach Programs.

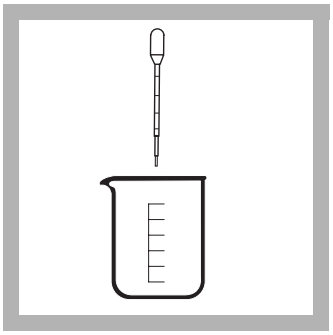
Select program

283 Lead, LeadTrak LR.

Touch **Start**.

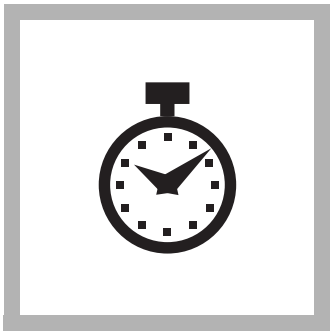


2. Fill a 100-mL plastic graduated cylinder with 100 mL of the sample. Pour the measured sample into a 250-mL plastic beaker.



3. Using a plastic 1-mL dropper, add 1.0 mL of pPb-1 Acid Preservative Solution to the sample and swirl to mix.

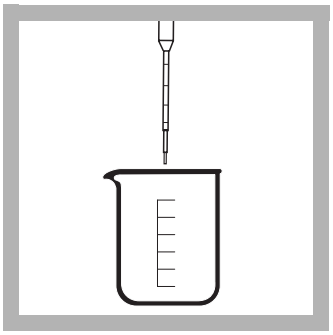
If the sample has been preserved previously with pPb-1 Acid Preservative at a ratio of 1.0 mL per 100 mL sample, omit steps 3 and 4.



4. Touch the timer icon.

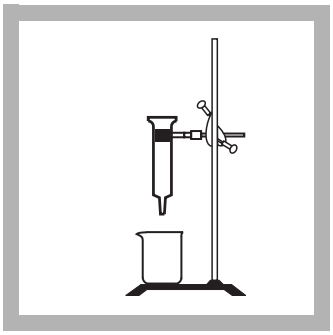
Touch **OK**.

A two-minute reaction period will begin.



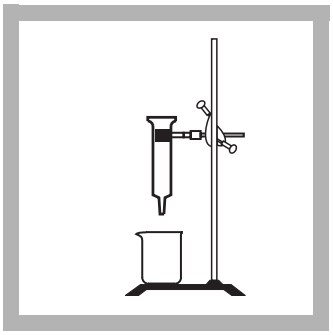
5. When the timer beeps, use a second 1-mL plastic dropper to add 2.0 mL of pPb-2 Fixer Solution. Swirl to mix.

Note: *Field samples that have been preserved with nitric acid or samples that have been digested may exceed the buffer capacity of the Fixer Solution. After step 5, check the pH of these samples and adjust with 5 N Sodium Hydroxide to a pH of 6.7–7.1 before proceeding with step 6.*



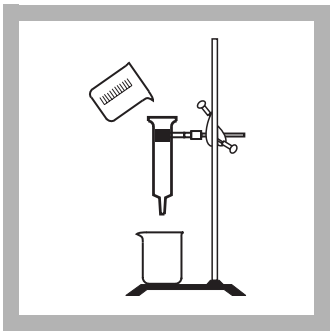
6. Mount a new Fast Column Extractor in a ring stand with a clamp. Place a 150-mL plastic beaker under the Extractor.

A Fast Column Extractor is included in the LeadTrak® Reagent Set. A new extractor is required for each test.



7. Soak the cotton plug with deionized water and compress it with the plunger. Remove the plunger. If the cotton plug moves up the column, push it back to the bottom with a clean, blunt rod.

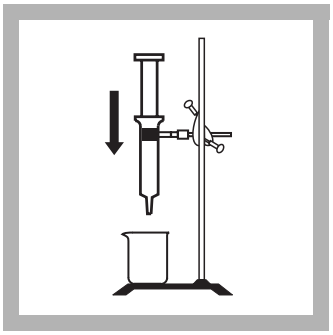
The cotton plug should fit snugly against the inner wall of the column.



8. Pour the prepared sample slowly into the center of the Column Extractor. Wait for the sample to flow through.

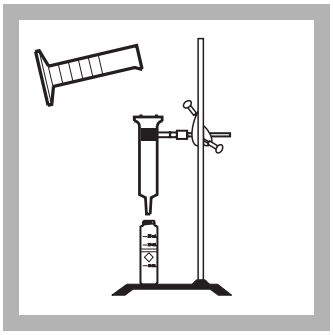
The sample solution should flow relatively slowly (2 drops per second) through the column.

Keep the level of the sample solution just above the cotton plug.



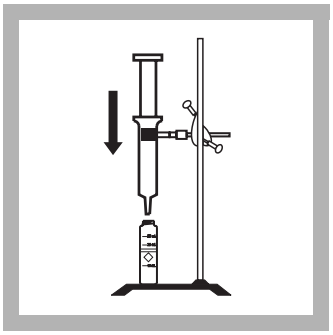
9. After the flow has stopped, fully compress the absorbent pad in the Extractor with the plunger. Discard the contents of the beaker. Slowly withdraw the plunger from the Extractor.

Note: *The absorbent pad should remain at the bottom of the Extractor when the plunger is removed. If the cotton plug moves up the column, push it back to the bottom with a clean, blunt rod.*



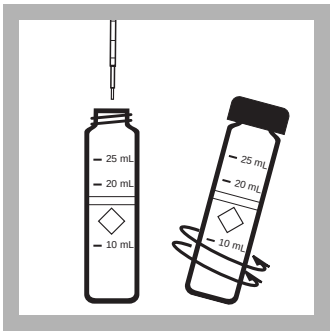
10. Place a clean, dry 25-mL sample cell under the Extractor. Using a 25-mL plastic graduated cylinder, add 25 mL of pPb-3 Eluant Solution to the Extractor.

Keep the level of the eluent solution just above the absorbent pad.

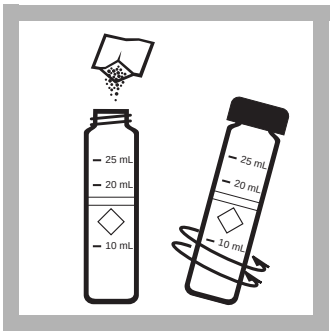


11. Allow the Eluant Solution to drip slowly from the Extractor.

After the flow has stopped, fully compress the absorbent pad. The volume in the sample cell should be 25 mL.

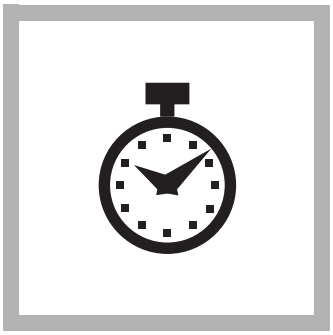


12. Using a 1-mL plastic dropper, add 1.0 mL of pPb-4 Neutralizer Solution to the cell. Swirl thoroughly to mix and proceed immediately to *step 13*.



13. Add the contents of one pPb-5 Indicator Powder Pillow to the sample and swirl thoroughly to mix.

The solution will turn brown.

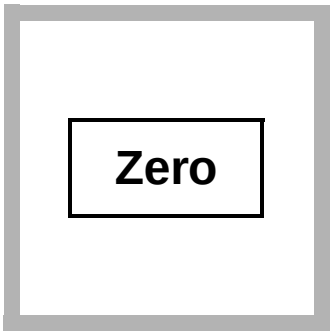


14. Touch the timer icon.

Touch **OK**.

A second two-minute reaction period will begin.

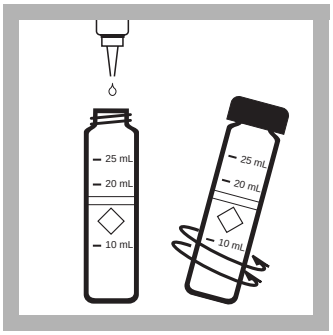
15. When the timer beeps, place the sample cell into the cell holder.



16. Touch **Zero**.

The display will show:

0 µg/L Pb



17. Remove the sample cell and add 6 drops of pPb-6 Decolorizer Solution to the cell. Swirl to mix thoroughly.

18. Place the sample cell into the cell holder.

Results will appear in µg/L Pb.

Interferences

Interference studies were conducted by preparing a known lead solution of approximately 25 µg/L as well as the potential interfering ion. The ion was said to interfere when the resulting lead concentration changed by ±10%. Samples containing levels exceeding these concentration values may be diluted 1:1 and re-analyzed. Multiply the value obtained by a factor of 2 to determine the lead present in the original sample.

Every effort has been made to prevent contamination in packaging the reagents. Use of black rubber stoppers, black dropper bulbs and droppers with inked graduations may contaminate the sample and should be avoided. Use the plastic droppers provided in the reagent set.

Acid-wash all glassware and plasticware to prevent sample contamination, especially if the previous sample had a high lead level (see *Apparatus and Sample Preparation*).

The Extractor plunger may be reused for more than one test but should be rinsed between uses.

Interfering Substance	Interference Levels and Treatments
Aluminum, Al^{3+}	0.5 mg/L
Ammonium, NH_4^+	500 mg/L
Barium, Ba^{2+}	6 mg/L
Calcium, Ca^{2+}	500 mg/L
Chloride, Cl^-	1000 mg/L
Copper, Cu^{2+}	2 mg/L
Fluoride, F^-	10 mg/L
Iron, Fe^{2+}	2 mg/L
Magnesium, Mg^{2+}	500 mg/L
Manganese, Mn^{2+}	05 mg/L
Nitrate, NO_3^-	1000 mg/L
Sulfate, SO_4^{2-}	1000 mg/L
Zinc, Zn^{2+}	1 mg/L

Apparatus and Sample Preparation

Because lead is very common to our environment, care must be taken to prevent sample contamination. Follow these steps for greatest test accuracy:

- Lead-free water is necessary to minimize sample contamination when rinsing apparatus or diluting sample. The water may be either distilled or deionized. If the water is obtained from a grocery store, verify the lead concentration is zero from the label. If the lead concentration is uncertain, determine the lead concentration with the LeadTrak test.
- Plastic or glass sample containers and lids may be checked for contamination by rinsing with 1 mL of pPb-1 Acid Preservative Reagent (Cat. No. 23685-31). Add 100 mL of lead-free water. After 24 hours, analyze this solution using the LeadTrak test to confirm the absence of lead.
- Rinse glassware used in this test with a small amount of dilute lead-free 0.1 N nitric acid or pPb-1 Acid Preservative Reagent followed by rinsing with lead-free water.
- pPb-5 Indicator may be rinsed from the glass sample cells with a few drops of pPb-1 Acid Preservative Reagent or a small amount of dilute lead-free nitric acid.
- Acidify solutions containing lead with Nitric Acid (Cat. No. 152-49) or pPb-1 to below pH 2 to prevent adsorption of lead onto the container walls. See *Sample Collection, Storage and Preservation*.

Sample Collection, Storage and Preservation

Samples may be collected either from household pipes (point-of-use) or from water sources. Preserved samples may be stored up to six months. Each sample type typically requires different sampling procedures. Consult with the appropriate regulatory agency in your area for more information about your specific sampling requirements.

Sampling for Lead Contamination in Household Pipes for Point-of-use Drinking Water

- The sample should be collected after sitting in pipes with no flow for 8 to 18 hours.
- Add 10 mL of pPb-1 Acid Preservative (Cat. No. 23685-31) to a one-liter bottle.
- Turn on tap and collect exactly the first liter of water in the bottle containing acid preservative.
- Cap and invert several times to mix.
- After two minutes the sample is ready for analysis. Steps 3 and 4 are skipped in the analysis procedure. Use 100 mL of this preserved sample directly in step 5.

Sampling for Lead Contamination from Drinking Water Sources Such as Well Water or Water from Main Supply Lines

- Add 10 mL of pPb-1 Acid Preservative (Cat. No. 23685-31) to a one-liter bottle.
- Turn on the tap for 3–5 minutes or until the water temperature has been stable for 3 minutes.
- Collect exactly one liter of water into the bottle containing the acid preservative.
- Cap and invert several times to mix.
- After two minutes the sample is ready for analysis. Steps 3 and 4 are skipped in the analysis procedure. Use 100 mL of this preserved sample directly in step 5.
- At least one liter should be collected to obtain a representative sample. If less than one liter is collected, use 1 mL of pPb-1 Acid Preservative per 100 mL of sample.
- If nitric acid is to be substituted for pPb-1 as a preservative or the sample is digested, the buffering capacity of the pPb-2 Fixer Solution (Cat. No. 23686-55) may be exceeded. Adjust the sample pH to 6.7–7.1 pH with 5 N Sodium Hydroxide (Cat. No. 2450-53) after step 6.

Reagent Blank Adjustment

The LeadTrak® program will allow a reagent blank value from -5 to +5 µg/L Pb to be automatically subtracted from the test result. When using the reagent blank adjustment feature, the concentration value displayed after zeroing should be 0 µg/L.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.

4. Open a container of 10-mg/L (10,000 µg/L) Lead Standard Solution.
5. Prepare three sample spikes. Fill three beakers with 100 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *46* for more information.

Standard Solution Method

Using Class A glassware, prepare a 100-µg/L lead working standard solution by pipetting 1.0 mL of Lead Standard Solution, 1000-mg/L, into a 100-mL volumetric flask. Use a TenSette® Pipet to add 0.2 mL of concentrated nitric acid to the flask. Dilute to the mark with lead-free deionized water. This makes a 10-mg/L working standard.

Pipet 10.00 mL of this working solution into a 1-liter plastic volumetric flask. Add 2.0 mL of concentrated nitric acid to the flask. Dilute to the mark with lead-free water. This 100-µg/L standard solution should be prepared immediately before use. Perform the LeadTrak® procedure as described above.

Alternatively, prepare a 100-µg/L lead standard solution by using a TenSette® Pipet to pipet 0.2 mL from a Lead Voluette® Ampule Standard Solution, 50-mg/L as Pb, into a 100-mL plastic volumetric flask. Add 0.2 mL of concentrated nitric acid, and dilute to volume with deionized water. Prepare this solution immediately before use.

To adjust the calibration curve using the reading obtained with the 100-µg/L standard solution:

1. Touch **Options** on the current program menu. Touch **Standard Adjust**.
2. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *49* for more information.

Method Performance

Precision

Standard: 50.0 µg/L Pb^{2+}

Program	Standard Deviation of 7 Replicate Standards
283	5 µg/L Pb^{2+}

See *Section 3.4.3 Precision* on page *53* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
50 to 150 µg/L	0.010	16 µg/L Pb^{2+}

See *Section 3.4.5 Sensitivity* on page *54* for more information.

Summary of Method

Acid soluble lead, as Pb^{2+}, in a potable water sample is first concentrated on a Fast Column Extractor. The lead is then eluted from the Extractor and determined colorimetrically with an indicator. Test results are measured at 477 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
LeadTrack® Reagent Set	1	20 tests / pkg	23750-00

Required Apparatus

Beaker, polypropylene, 150-mL	1	each	1080-44
Beaker, polypropylene, 250-mL	1	each	1080-46
Clamp, two-prong extension	1	each	21145-00
Clamp holder	1	each	326-00
Clippers, for opening powder pillows	1	each	936-00
Cylinder, graduated, polypropylene, 25-mL	1	each	1081-40
Cylinder, graduated, polypropylene, 100-mL	1	each	1081-42
Dropper, 0.5 & 1.0 mL marks		20 / pkg	21247-20
Sample Cells, 10-20-25 mL, w / cap	2	6 / pkg	24019-06
Support, ring stand	1	each	563-00

Digestion Reagents and Required Standards

Lead Standard Solution, 1000-mg / L as Pb	100 mL	12796-42
Lead Standard Solution, 50-mg / L, 10-mL Voluette® Ampules	16 / pkg	14262-10
Lead Standard Solution, 10-mg / L	25 mL	23748-20
Nitric Acid, ACS	500 mL	152-49
Water, deionized	4 liters	272-56

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Method 10065

Cold Vapor Mercury Concentration Method*

(0.1 to 2.5 µg/L)

Scope and Application: For water, wastewater, and seawater

* Patent no. 5,733,786

Tips and Techniques

- Perform Phase 1 of the procedure in a fume hood. Toxic chlorine or other gases may be produced.
- Use dedicated digestion glassware and sample cells for this procedure.
- Determine a reagent blank for each new lot of reagent by running the entire procedure, including the digestion, using one liter of deionized water instead of sample. Add the same amount of potassium permanganate as required by the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.

Phase 1: Sample Digestion

(Must be done in a fume hood – toxic gases may be produced!)

1. Transfer one liter of the sample to a 2000-mL Erlenmeyer flask. Add a 50-mm magnetic stir bar to the sample. Place the flask on a magnetic stirring hot plate and begin stirring.

2. Add 50 mL of concentrated sulfuric acid to the sample.

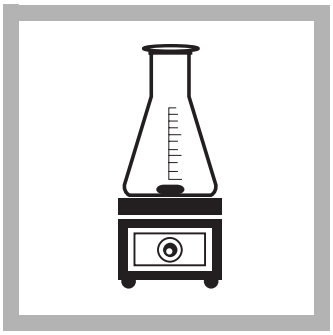
3. Add 25 mL of concentrated nitric acid to the sample.

4. Add 4.0 g of potassium persulfate to the sample. Stir until dissolved.

Alternatively, add one 5-gram measuring scoop of potassium persulfate to the sample.

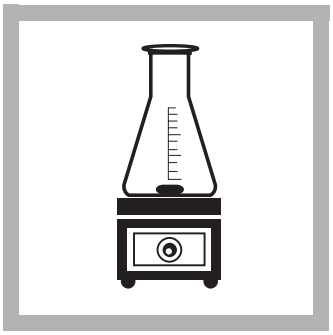
5. Add 7.5 g of potassium permanganate to the sample. Stir until dissolved.

Alternatively, add a 10-gram measuring scoop of potassium permanganate to the sample.



6. Cover the flask with a watch glass. Begin heating the sample to a temperature of 90 °C after the reagents have dissolved. **Do not boil.**

Note: *For a mercury standard or reagent blank in distilled water, the heat step is not necessary.*

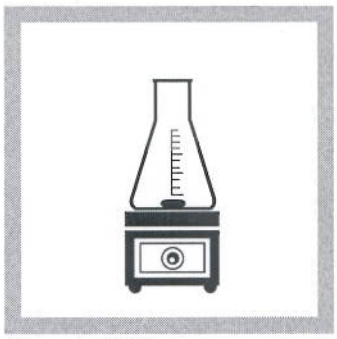


7. Continue to stir and heat the sample at 90 °C for two hours.

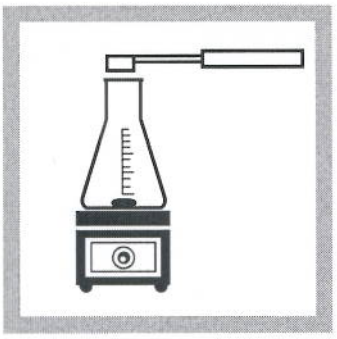
The solution must remain dark purple throughout the entire digestion. Some samples, such as sea waters, industrial effluents or other samples high in organic matter or chloride concentration, require additional permanganate. It may be difficult to see a dark purple color if the sample contains black / brown manganese dioxide precipitate. Add more potassium permanganate if the solution is not dark purple.

8. Cool the digested sample to room temperature.

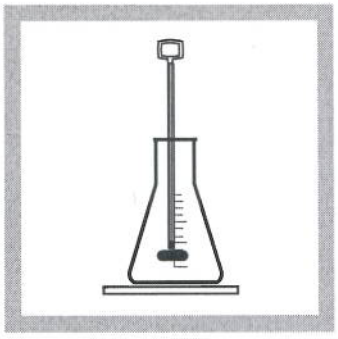
A brown / black precipitate of manganese dioxide may settle during cooling. If the digested sample does not have a purple color, the digestion may be incomplete. Add more potassium permanganate. Return the sample to the magnetic stirring hot plate and continue the digestion until the purple color persists.



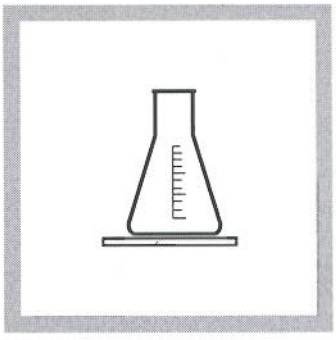
9. Return the cool, digested sample to the cool, magnetic stirring hot plate. Turn on the stirrer.



10. Using a 0.5-g measuring spoon, add 0.5 g additions of hydroxylamine-hydrochloride until the purple color disappears. Wait 30 seconds after each addition to see if the purple disappears. Add hydroxylamine-hydrochloride until all manganese dioxide is dissolved.

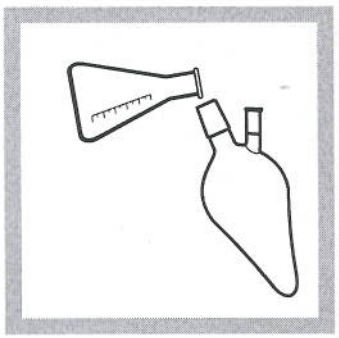


11. Remove the stir bar.

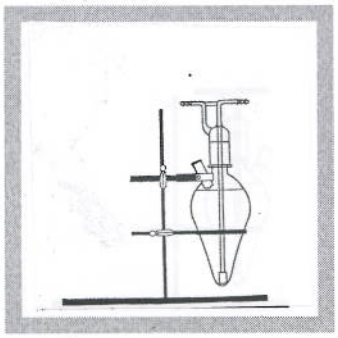


12. The digested sample is now ready for processing by cold vapor separation and preconcentration. Proceed to Phase 2.

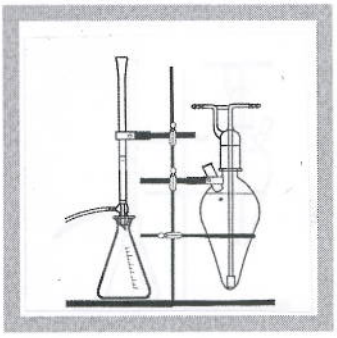
Phase 2: Cold Vapor Separation and Preconcentration of Mercury



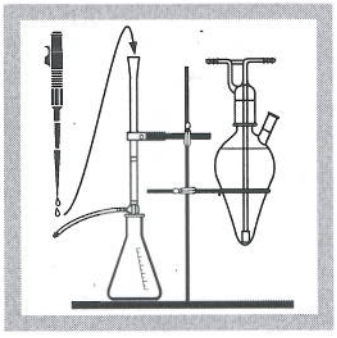
1. Transfer the digested sample to the Cold Vapor Gas Washing Bottle. (The volume of the digested sample should contain 0.1 to 2.5 µg Hg.)



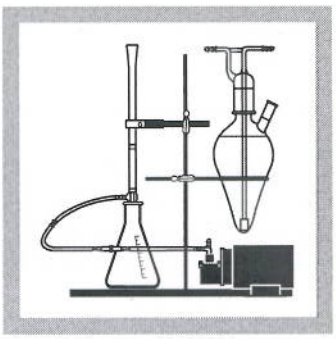
2. Set the Gas Washing Bottle in the support ring. Clamp the small neck of the Gas Washing Bottle Place the top on the Gas Washing Bottle. Wait until step 9 to connect the mercury absorber column to the Gas Washing Bottle.



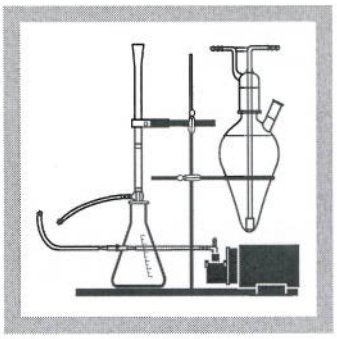
3. Connect the 100-mL Erlenmeyer flask with a ground glass joint to the mercury absorber column.



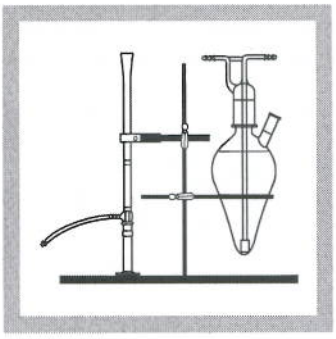
4. Pipet 8 mL of HgEx Reagent B into the Mercury Absorber column.



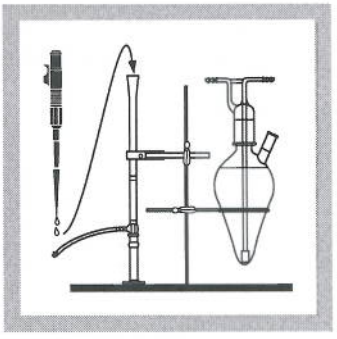
5. Install the Mercury Scrubber in the tubing Between the Mercury Absorber Column and the vacuum pump. Connect power to the vacuum pump and apply vacuum to the Mercury Absorber Column. Draw most of the HgEx Reagent B into the Erlenmeyer flask.



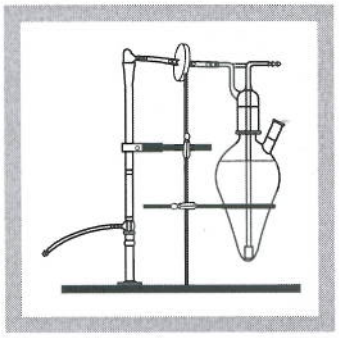
6. Disconnect the vacuum using the quick disconnect when HgEx Reagent B begins to drip from the inner delivery tube on the Mercury Absorber Column (about 10 seconds after starting the vacuum). Do not draw enough air through the column to begin drying the packing.



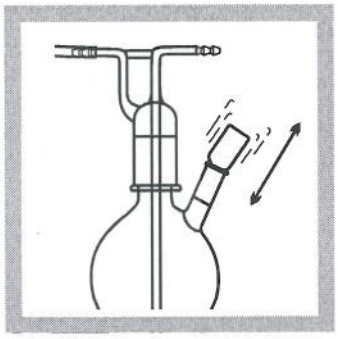
7. Remove the 100-mL Erlenmeyer flask from the Mercury Absorber Column. Replace it with the 10-mL Distilling Receiver.



8. Pipet 2 mL of HgEx Reagent C into the Mercury Absorber Column.

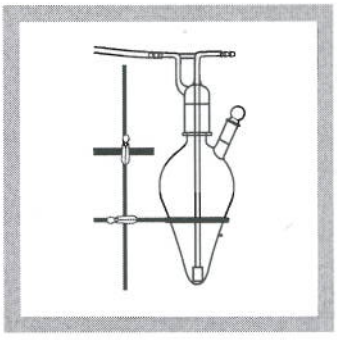


9. Connect the Mercury Absorber Column to the Gas Washing Bottle using the glass elbow. Install the Acro 50 vent filter in the tubing, with the printed side toward the Gas Washing Bottle.

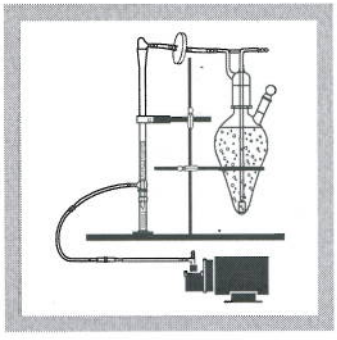


10. Shake an ampule of HgEx Reagent A to suspend undissolved reagent.

Open the ampule and gently shake the contents into the Gas Washing Bottle through the side neck.



11. Stopper the side neck on the Glass Washing Bottle.



12. Reconnect the vacuum to the Mercury Absorber Column using the quick disconnect. The vacuum will pull HgEx Reagent C through the Mercury Absorber Column packing and into the 10-mL receiver. Air bubbles should be produced at the gas dispersion tube in the Gas Washing Bottle. Perform steps 13–14 immediately.

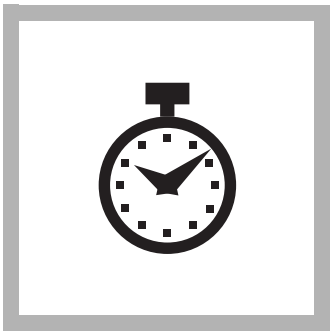
13. Touch

Hach Programs.

Select program

312 Mercury, Cold Vapor.

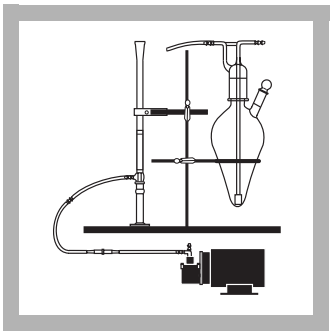
Touch **Start**.



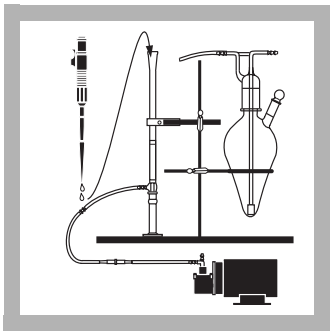
14. Touch the timer icon. Touch **OK**.

A five-minute reaction period will begin. Let the solution bubble for this period.

Air flow rate through the Gas Washing Bottle should be between 1–5 L / min. Allow more bubbling time for lower air flow rates. For example, if the air flow rate is 1 L / min., let the solution bubble for 10 min.

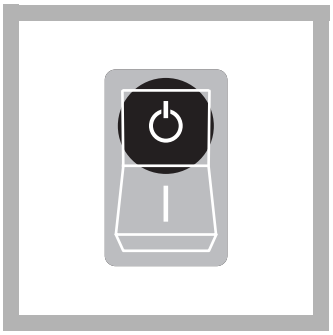


15. After the timer beeps, remove the glass elbow from the top of the Mercury Absorber Column. Keep the vacuum pump on.



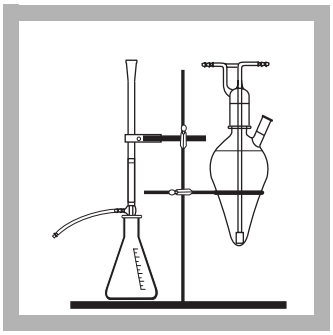
16. Pipet 8 mL of HgEx Reagent B into the Mercury Absorber Column to elute the captured mercury.

Continue to apply vacuum to pull the HgEx Reagent B into the Distilling Receiver.

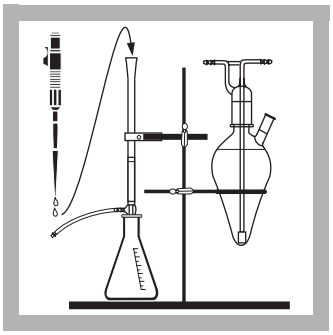


17. Turn off, or disconnect power to the vacuum pump when the volume in the Distilling Receiver reaches the 10 mL mark.

If necessary, the volume in the Distilling Receiver may be brought up to 10 mL with HgEx Reagent B. To avoid low volumes in the future, disconnect the vacuum a little sooner in step 6. This leaves more HgEx Reagent B in the packing of the Mercury Absorber Column.



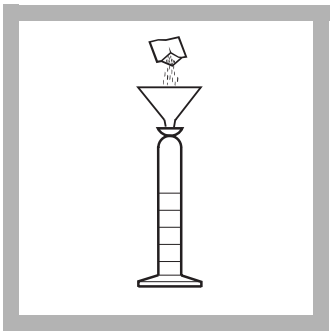
18. Remove the distilling Receiver from the Mercury Absorber Column. Reconnect the 100-mL Erlenmeyer flask to the column.



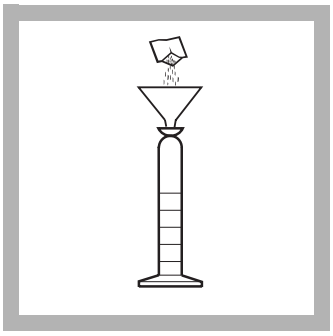
19. Pipet 3 mL of HgEx Reagent B into the Mercury Absorber Column without applying vacuum. This keeps the absorber packing wet between tests.

The Mercury Absorber Column eluate in the Distilling Receiver is ready for analysis. Proceed to Phase 3.

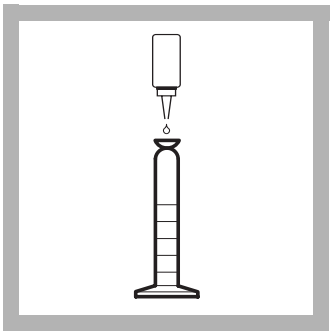
Phase 3: Colorimetric Analysis



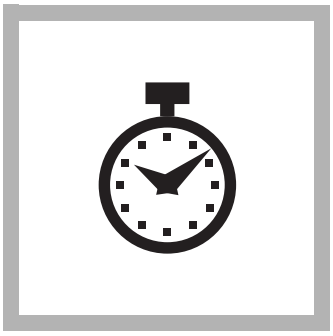
1. Using the funnel provided, add the contents of one HgEx Reagent 3 foil pillow to the eluate in the Distilling Receiver. Stopper the receiver. Invert to dissolve the reagent.



2. Add the contents of one HgEx Reagent 4 foil pillow to the Distilling Receiver using the funnel provided. Stopper the receiver. Invert to dissolve the reagent.



3. Add 8 drops of HgEx Reagent 5 to the Distilling Receiver. Stopper the Receiver. Invert to mix.



4. Touch the timer icon.

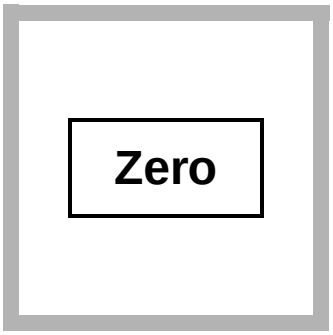
Touch **OK**.

A two-minute reaction period will begin.

5. During the reaction period, transfer the solution to a sample cell. Wipe the sample cell sides with a clean tissue.

Do not cap the sample cell.

6. After the timer beeps, place the prepared sample into the cell holder.



7. Touch **Zero**.

The display will show:

0.1 µg/L Hg

(This program uses a non-zero intercept.)

8. Remove the cell from the cell holder. Add the contents of one HgEx Reagent 6 foil pillow to the solution. Swirl or invert the cell until the reagent is completely dissolved. Immediately go to step 9.

Do not use the funnel to add HgEx Reagent 6 to the sample cell. Any HgEx Reagent 6 in the funnel will make mercury undetectable in subsequent tests.

9. Return the sample cell to the cell holder.

Results will appear in µg/L Hg.

Interferences

Standards were used to prepare a single test solution with the following matrix. A second test solution containing only mercury at the same concentration was prepared as the control. The two solutions were digested then analyzed concurrently. There was no interference from the matrix of the test solution at the concentrations listed:

In addition, no interference occurred with a test solution containing 1000 mg/L Na^+, 1000 mg/L K^+, 1000 mg/L Mg^{2+}, and 400 mg/L Ca^{2+}.

Ion or Substance	Concentration
Ag^+	7 mg/L Ag^+
Al^{+3}	10 mg/L Al^{+3}
Au^{+3}	500 µg/L Au^{+3}
Cd^{+2}	10 mg/ L Cd^{+2}
Co^{+2}	10 mg/L Co^{+2}
Cr^{+6}	10 mg/L Cr^{+6}
Cu^{+2}	10 mg/L Cu^{+2}
F^-	1.0 mg/L F^-
Fe^{+2}	100 mg/L Fe^{+2}
Hg^{+2}	1 µg/L Hg^{+2}
Mo^{+6}	10 mg/L Mo^{+6}
Ni^{+2}	10 mg/L Ni^{+2}
NO_3^-–N	50 mg/L NO_3^-–N
Pb^{2+}	10 mg/L Pb^{2+}
SiO_2	100 mg/L SiO_2
Zn^{+2}	10 mg/L Zn^{+2}

Sample Collection and Preservation

Collect 1000 mL of sample in an analytically clean, glass or polyethylene terephthalate (PET) container. Add 10 mL of concentrated hydrochloric acid to preserve the sample before sample collection. Fill the container completely full to minimize air space when closed. Close a glass container with a ground glass stopper. Close a PET container with a PET cap or a polypropylene cap (no liner).

Store aqueous samples at 2–6 °C. Acid-preserved samples are stable for at least 6 months.

Accuracy Check

Standard Additions Method

1. Prepare a 10.0-mg/L Mercury Standard Solution as described under *Standard Solution Method, Step 3a,* below.
2. Use a TenSette® Pipet to add 0.10 mL of the 10.0-mg/L Mercury Standard Solution to the purged solution in the Gas Washing Bottle after an analysis has been performed. Immediately stopper the Gas Washing Bottle.
3. Begin at *step 3* of Phase 2. Follow the procedure steps.
4. Test the eluate as described in Phase 3. The displayed concentration should be 0.9–1.1 µg/L Hg.

Standard Solution Method

1. Transfer 800 mL of deionized water into the Gas Washing Bottle.
2. Add 50 mL of concentrated sulfuric acid and 25 mL of concentrated nitric acid to the water. Swirl to mix.
3. Prepare a 0.1-mg/L mercury standard solution by serially diluting a 1000-mg/L Mercury Standard Solution:
   a. To make a 10.0 mg/L standard, add 1.0 mL of concentrated nitric acid to a 500-mL volumetric flask. Dilute 5.00 mL of a 1000-mg/L standard to 500 mL with deionized water. Mix well.
   b. To make a 1.0-mg/L standard solution, add 0.2 mL of concentrated nitric acid to a 100-mL volumetric flask. Dilute 10.0 mL of the 10.0-mg/L standard to 100 mL with deionized water. Mix well.
   c. To make a 0.1-mg/L standard solution, add 0.2 mL of concentrated nitric acid to a 100-mL volumetric flask. Dilute 10.00 mL of the 1.0-mg/L solution to 100 mL with deionized water. Mix well.
4. Pipet 10.0 mL of the 0.1-mg/L mercury standard solution into the Gas Washing Bottle. Swirl to mix.
5. Begin at *step 2* of Phase 2. Follow the procedure steps.
6. Test the eluate as described in Phase 3. The displayed concentration should be 0.9–1.1 µg/L Hg.

System Start Up

Hach recommends that the analyst perform a few analyses on mercury standards and blanks for system equilibration before beginning sample testing. This allows the system to stabilize before processing samples.

Startup Standard

Test a mercury standard solution by following the procedure under *Accuracy Check* using the *Standard Solution Method*. Continue with *step 1* (below) if the value is not within specified limits.

1. Pipet 10.0 mL of the 0.1-mg/L mercury standard solution into the purged solution in the Gas Washing Bottle. Immediately stopper the Gas Washing Bottle.
2. Begin at *step 3* of Phase 2. Follow the procedure steps.
3. Test the eluate as described in Phase 3. The displayed concentration should be 0.9–1.1 µg/L Hg. Repeat *steps 1–3* if the value is not within these limits.

Startup Blank

Run a system blank by using the purged solution in the Gas Washing Bottle after a satisfactory test of the Startup Standard has been completed.

1. Leave the purged solution in the Gas Washing Bottle. Do not add an aliquot of mercury standard.
2. Begin at *step 3* of Phase 2. Follow the procedure steps.
3. Test the eluate as described in Phase 3. The displayed concentration should be ≤ 0.2 µg/L Hg. Repeat the *Startup Blank* procedure until a reproducible value is obtained.

Method Performance

Precision

Standard: 1.40 µg/L Hg

Program	95% Confidence Limits
312	1.3–1.5 µg/L Hg

See *Section 3.4.3 Precision* on page *53* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.03 µg/L Hg

See *Section 3.4.5 Sensitivity* on page *54* for more information.

Storage and Maintenance of the Cold Vapor Mercury Apparatus

Storage

Store the apparatus as follows for fastest system stabilization and greatest sensitivity:

- Store the Gas Washing Bottle filled with deionized water containing 15 mL of concentrated sulfuric acid. Seal the bottle with the Gas Washing Bottle stopper and top.
- Store the Mercury Absorber Column with the packing wetted with HgEx Reagent B. The erlenmeyer flask should be kept attached underneath the column. The top of the Mercury Absorber column should be attached to the Gas Washing Bottle with the glass elbow as in the procedure.

Glassware Care

Hach recommends using dedicated glassware and sample cells because of the sensitivity of this procedure. Thoroughly clean the glassware and sample cells between tests. After washing, rinse with 1:1 hydrochloric acid solution, then rinse several times with deionized water.

Maintaining the System

- With proper care and storage, the Mercury Absorber Column may be used an unlimited number of times.
- Replace the Mercury Scrubber in the air trap housing at least once for every reagent set used.
- Moisture build up on the Gas Washing Bottle side of the Acro 50 Vent Filter will reduce the purging air flow rate. If this occurs replace the filter or dry it in an oven at 110 °C.

Summary of Method

The sample is digested to convert all forms of mercury in the sample to mercuric (Hg^{2+}) ions. The mercuric ions in the digested sample are converted to mercury vapor in a semi-closed system. The vapor is carried into a chemically activated absorber column by ambient air where the mercury vapor is converted to mercuric chloride.

The mercuric chloride is eluted off the column and a sensitive indicator is added. The instrument is zeroed using the absorbance peak of the unreacted indicator. A complexing agent is added to break the mercury:indicator complex. The increase in unreacted indicator causes an increase in absorbance s proportional to the amount of mercury in the original sample. Test results are measured at 412 nm.

Safety

Wear personal protective equipment such as safety glasses with side shields, or a face shield to protect your eyes. Use other protective equipment as necessary (such as a fume hood) to avoid chemical exposure. Perform all steps exactly as prescribed in the procedure.

Pollution Prevention and Waste Management

Proper management and disposal of waste is the responsibility of the waste generator. Hach Company provides waste disposal information as a guideline only. It is up to the generator to arrange for proper disposal and comply with applicable local, state, and federal regulations governing waste disposal. Hach Company makes no guarantees or warranties, express or implied, for the waste disposal information represented in this procedure.

1. Dispose of the solution in the Gas Washing Bottle by neutralizing the solution to a pH of 6–9 and flushing to the sanitary sewer with water for several minutes.
2. The mercury contained in one liter of sample is concentrated by a factor of 100 by the Mercury Absorber Column. Mercury analysis within the range of the test may produce a solution in the sample cell that is above the RCRA Toxicity Characteristic limit of 0.20 mg/L Hg. The sample cell will contain 0.25 mg/L mercury if the original sample was at 2.5 µg/L mercury (the upper limit of the test range). Dispose of the solution in the sample cell as a hazardous waste if the test result was over 2 µg/L mercury in the original sample. Otherwise, pour the solution into the sanitary sewer and flush with water for several minutes.
3. The mercury scrubber will capture mercury vapor if the Mercury Absorber Column is not properly activated using HgEx Reagent B and HgEx Reagent C. In addition, mercury is also captured if the capacity of the Absorber Column is exceeded. If the Mercury Scrubber has captured mercury vapor, it must be disposed of according to applicable regulations.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Cold Vapor Mercury Reagent Set (25 tests)			26583-00
Includes:			
HgEx™ Reagent A, Stannous Sulfate Solution, 20-mL ampules	1	25/pkg	26588-25
HgEx™ Reagent B, Sulfuric Acid Solution	19 mL	500 mL	26589-49
HgEx™ Reagent C, Sodium Hypochlorite Solution	2 mL	55 mL	26590-59
HgEx™ Reagent 3, Alkaline Reagent Powder Pillows	1 pillow	25/pkg	26584-48
HgEx™ Reagent 4, Indicator Powder Pillows	1 pillow	25/pkg	26585-48
HgEx™ Reagent 5, Sodium Hydroxide Solution	8 drops	10 mL SCDB	26586-36
HgEx™ Reagent 6, Complexing Reagent Powder Pillows	1 pillow	25/pkg	26587-48
Mercury Scrubber	2/reagent set	2/pkg	26558-00

Mercury

Required Digestion Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Hydroxylamine Hydrochloride	varies	113 g	246-14
Nitric Acid, ACS	25 mL	500 mL	152-49
Potassium Permanganate, ACS	varies	454 g	168-01H
Potassium Persulfate, ACS	4.0 g	454 g	26175-01
Sulfuric Acid, ACS, concentrated	50 mL	4 kg	979-09

Required Apparatus

Cold Vapor Mercury Apparatus Set			26744-00
Acro 50 Vent Filter	1	18 / pkg	26833-18
Air Trap Holder Assembly	1	each	26639-00
Ampule Breaker	1	each	25640-00
Breaker / Capper Tool for Mercury Scrubber	1	each	26640-00
C-flex Tubing, 0.25-inch ID, white	4 ft	25 ft	23273-67
Clamp for Mercury Absorber Column	1	each	26562-00
Clamp Holder	2	each	326-00
Cylinder, graduated, 50-mL	1	each	508-41
Distilling Receiver, 10-mL	1	each	26554-38
Flask, Erlenmeyer, 100-mL	1	each	26553-42
Funnel, micro	1	each	25843-35
Gas Washing Bottle, 1200-mL	1	each	26622-00
Glass Elbow, 90-degree, with hose adapter	1	each	26552-00
Mercury Absorber Column	1	each	26555-10
Pipet, TenSette®, 0.1 to 1.0 mL		each	19700-01
Pipet, TenSette®, 1.0 to 10.0 mL		each	19700-10
Pipet Tips, for 19700-01 TenSette® Pipet		50 / pkg	21856-96
Pipet Tips, for 19700-10 TenSette® Pipet		50 / pkg	21997-96
Sample Cells, 10-mL, w / cap	2	6 / pkg	24276-06
Support Ring for Gas Washing Bottle	1	each	26563-00
Stopper, for Distilling Receiver	1	each	26559-00
Stopper, for Gas Washing Bottle	1	each	26623-00
Support, Base and Rod	1	each	329-00
Tubing Quick Disconnect, HDPE	1	12 / pkg	14810-00
Vacuum Pump, with fittings, 115 VAC		each	26557-00
Vacuum Pump, with fittings, 230 VAC		each	26557-02

Required Digestion Apparatus

Flask, Erlenmeyer, 2000-mL	1	each	24894-54
Hot Plate / Stirrer, 120 VAC	1	each	23442-00
Hot Plate / Stirrer, 240 VAC	1	each	23442-02
Spoon, measuring, 0.5-g	1	each	907-00
Stir Bar	1	each	20953-55
Thermometer, -20 to 110 °C	1	each	566-01
Watch Glass, Pyrex, 65-mm	1	each	578-67

Required Standards

Mercury Standard Solution, 1000-mg / L Hg (NIST)		100 mL	14195-42
Water, deionized		4 L	272-56

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WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Method 10020 **Chromotropic Acid Method**

Test 'N Tube™ Vials **HR (0.2 to 30.0 mg/L NO_3^-–N)**

Scope and Application: For water and wastewater

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water (nitrate-free) in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- This test is technique-sensitive. Invert the vials as described here to avoid low results: Hold the vial in a vertical position with the cap pointing up. Turn the vial upside-down. Wait for all of the solution to flow down to the cap. Pause. Return the vial to an upright position. Wait for all the solution to flow to the bottom of the vial. This process equals one inversion.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Test 'N Tube Method 10020

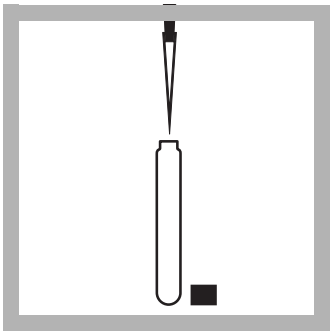
1. Touch

Hach Programs.

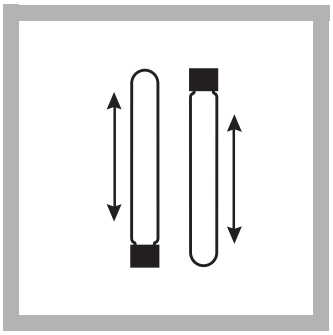
Select program

344 N, Nitrate HR TNT.

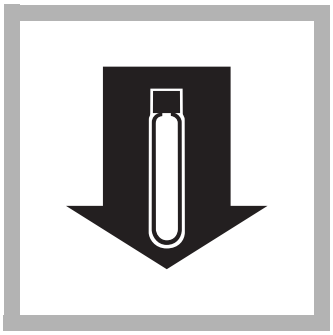
Touch **Start**.



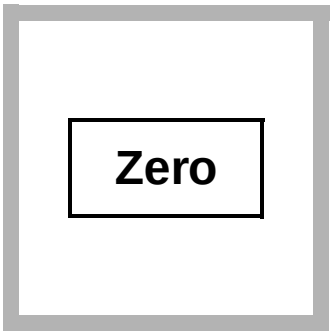
2. Remove the cap from a NitraVer X Reagent A Test 'N Tube vial and add 1.00 mL of sample (this is the blank).



3. Cap the tube and invert ten times to mix.



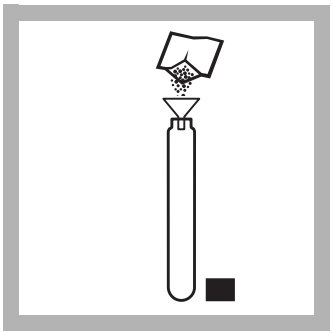
4. Wipe the blank and place it into the cell holder.



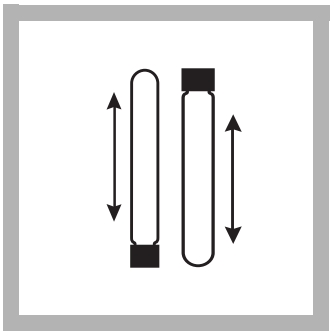
5. Touch **Zero**.

The display will show:

0.0 mg/L NO_3^-–N

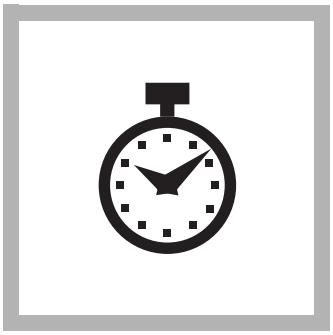


6. Remove the vial from the instrument. Using a funnel, add the contents of one NitraVer X Reagent B Powder Pillow to the vial.



7. Cap and invert ten times to mix (this is the *prepared sample*).

Some solid matter will not dissolve.

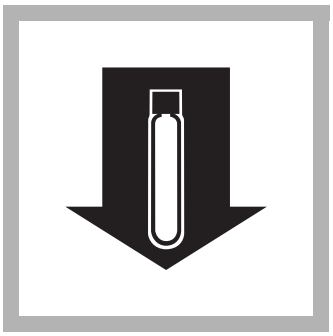


8. Touch the timer icon.

Touch **OK**.

A five-minute reaction period will begin. Do not invert the vial again.

A yellow color will develop if nitrate is present.



9. Within five minutes after the timer beeps, wipe the prepared sample and place it into the cell holder. Results will appear in mg/L NO_3^-–N.

Interferences

Interfering Substance	Interference Levels and Treatments
Barium	A negative interference at concentrations greater than 1 mg/L.
Chloride	Does not interfere below 1000 mg/L.
Nitrite	A positive interference at concentrations greater than 12 mg/L. Remove nitrite interference up to 100 mg/L by adding 400 mg (one full 0.5 g Hach measuring spoon) of Urea (Cat. No. 11237-26) to 10 mL of sample. Swirl to dissolve. Proceed with the nitrate test as usual.
Copper	Positive at all levels.

Sample Collection, Preservation, and Storage

Collect samples in clean plastic or glass bottles. Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. For longer storage periods (up to 14 days), adjust sample pH to 2 or less with Concentrated Sulfuric Acid, ACS (about 2 mL per liter) (Cat. No. 979-49). Sample refrigeration is still required.

Before testing the stored sample, warm to room temperature and neutralize with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-26).

Do not use mercury compounds as preservatives.

Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *23*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a High Range Nitrate Nitrogen Voluette® Ampule Standard, 500 mg/L NO_3^-–N.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *26* for more information.

Standard Solution Method

Use a 10.0-mg/L Nitrate Nitrogen Standard Solution to check test accuracy.

See *Section 3.2.1 Standard Solutions* on page *25* for more information.

Method Performance

Precision

Standard: 10.0 mg/L NO_3^-–N

Program	95% Confidence Limits of Distribution
344	9.5–10.5 mg/L NO_3^-–N

See *Section 3.4.3 Precision* on page *33* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.2 mg/L NO_3^-–N

See *Section 3.4.5 Sensitivity* on page *34* for more information.

Summary of Method

Nitrate in the sample reacts with chromotropic acid under strongly acidic conditions to yield a yellow product with a maximum absorbance at 410 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Test 'N Tube NitraVer® X Nitrate Reagent Set (50 tests)			26053-45

Required Apparatus

Funnel, micro, poly	1	each	25843-35
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	varies	50/pkg	21856-96
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
Test Tube Rack, cooling	1–3	each	18641-00

Required Standards

Nitrate Nitrogen Standard Solution, 10-mg/L N		500 mL	307-49
Nitrate Nitrogen Standard Solution, Voluette® Ampule, 500-mg/L N		16/pkg	14260-10
Wastewater Effluent Standard, for mixed parameters NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC		500 mL	28332-49
Water, deionized		4 liters	272-56

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Nitrite

Method 10019

Diazotization Method

Test ‘N Tube™ Vials

LR (0.003 to 0.500 mg/L NO_2^-–N)

Scope and Application: For water, wastewater, and seawater

Test Preparation

Before starting the test:

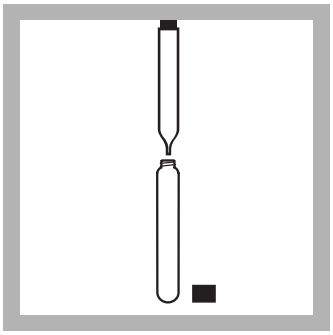
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

Collect the following items:	**Quantity**
Test ‘N Tube™ NitriVer® 3 Nitrite Reagent Set	1
Pipet, TenSette®, 1.0 to 10.0 mL, plus tips	1

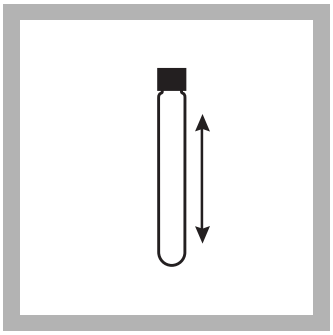
Note: *Reorder information for consumables and replacement items is on page 3.*

Test ‘N Tube — Method 10019

1. Select the test.



2. Fill a Test ‘N Tube NitriVer® 3 Nitrite vial with 5 mL of sample.

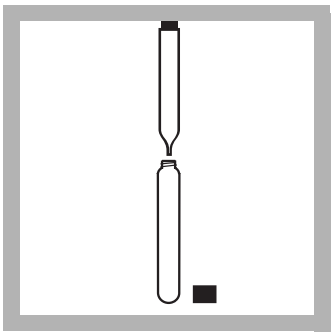


3. Prepared Sample: Cap and shake to dissolve the powder.

A pink color will develop if nitrite-nitrogen is present.

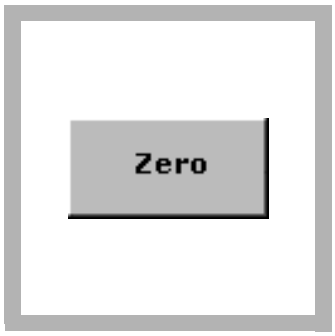
4. Press **TIMER>OK**.

A 20-minute reaction period will begin.



5. Blank Preparation: When the timer expires, fill an empty Test 'N Tube™ vial with 5 mL of sample.

6. Wipe the blank and insert it into the 16-mm round cell holder.



7. Press **ZERO**.

The display will show: 0.000 mg/L NO_2^-–N

8. Insert the prepared sample cell into the 16-mm round cell holder.

Results are in mg/L NO_2^-–N.

Interferences

Table 1 Interfering Substances and Levels

Interfering Substance	Interference Levels and Treatments
Antiminous ions	Interfere by causing precipitation
Auric ions	Interfere by causing precipitation
Bismuth ions	Interfere by causing precipitation
Chloroplatinate ions	Interfere by causing precipitation
Cupric ions	Cause low results
Ferric ions	Interfere by causing precipitation
Ferrous ions	Cause low results
Lead ions	Interfere by causing precipitation
Mercurous ions	Interfere by causing precipitation
Metavanadate ions	Interfere by causing precipitation
Nitrate	Very high levels of nitrate (>100 mg/L nitrate as N) appear to undergo a slight amount of reduction to nitrite, either spontaneously or during the course of the test. A small amount of nitrite will be found at these levels.
Silver ions	Interfere by causing precipitation
Strong oxidizing and reducing substances	Interfere at all levels

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Store at 4 °C (30 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test.

Accuracy Check

Standard Solution Method
Preparing nitrite standards is difficult. Use the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*, Method 4500-NO2 B. Prepare a 0.300-mg/L standard. Perform the nitrite test on the standard solution.

Method Performance

Precision
Standard: 0.300 mg/L NO_2^-–N

Program	95% Confidence Limits of Distribution
345	0.294–0.306 mg/L NO_2^-–N

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.003 mg/L NO_2^-–N

Summary of Method

Nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present. Test results are measured at 507 nm.

Consumables and Replacement Items

Required Reagents

Description	Quantity/Test	Unit	Cat. No.
Test 'N Tube™ NitriVer® 3 Nitrite Reagent Set	1	50/pkg	26083-45

Required Apparatus

Description	Quantity/Test	Unit	Cat. No.
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	19700-10
Pipet Tips, for TenSette Pipet 19700-10	varies	50/pkg	21997-96

Recommended Standards and Apparatus

Description	Unit	Cat. No.
Handbook, Standard Methods for the Examination of Water and Wastewater	each	22708-00
Pipet Tips, for TenSette Pipet 19700-10	250/pkg	21997-25
Sodium Nitrite, ACS	454 g	2452-01
Water, deionized	4 L	272-56

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Updated April 05 Edition 1

Selenium

Method 8194

Diaminobenzidine Method[1] (0.01 to 1.00 mg/L)

Scope and Application: For water and wastewater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test Preparation

Before starting the test:

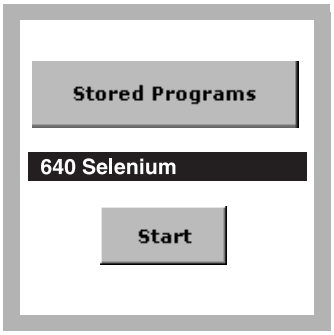
Distillation is required for determining total selenium. See Distillation on page 5 at the end of the procedure. Use the distillate as the sample in step 3.
Acetone[1] is a suitable solvent for removing toluene from glassware after results are measured.
Toluene (F005) solutions are regulated as hazardous waste by the Federal RCRA. Do not pour these materials down the drain. Water saturated with toluene, toluene solutions, and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal with laboratory solvent wastes. Refer to the current MSDS for safe disposal and handling information.
If there are visible water bubbles on the bottom of the cell, decant the top portion into a clean, dry 25-mL cell prior to reading the sample.

Collect the following items:	**Quantity**
Buffer Solution, sulfate type, pH 2.0	10 mL
Cotton Ball	1
Cylinder, Graduated: 50- and 100-mL	1 of each
Diaminobenzidine, tetrahydrochloride	0.1 g
Distillation Reagents and Apparatus (page 8)	—
Dropper, 0.5 and 1.0 mL marks, one glass and one plastic	1 of each
Flask, Erlenmeyer, 500-mL	2
Funnel, separation	2
Hot Plate, 4-inch diameter	1
Pipet, volumetric, 5-mL, plus safety bulb filler	1
Potassium Hydroxide Standard Solution, 12 N	4 mL
Ring support (3-inch) and stand	1
Sample Cells, 1-inch square glass, 25-mL	2
Spoons, measuring, 0.2 and 0.05 g	1 of each
TitraVer® Hardness Reagent	0.4 g
Toluene, ACS	60 mL
Water, Deionized	100 mL

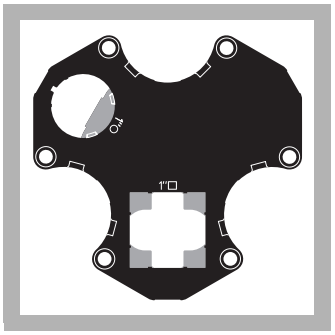
Note: *Reorder information for consumables and replacement items is on page 7.*

[1] See Optional Reagents and Apparatus on page 8.

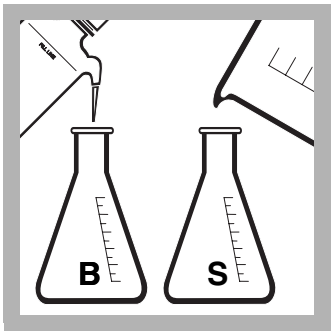
Diaminobenzidine Method 8194



1. Select the test.

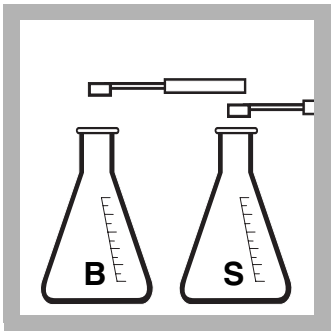


2. Insert the Multi-cell Adapter with the 1-inch square cell holder facing the user.

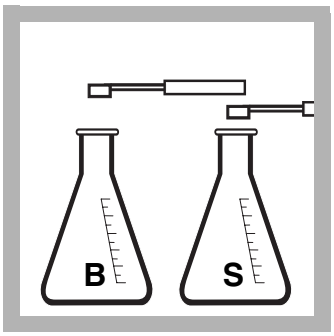


3. Measure 100 mL of deionized water into a 500-mL Erlenmeyer flask. Label the flask “blank”.

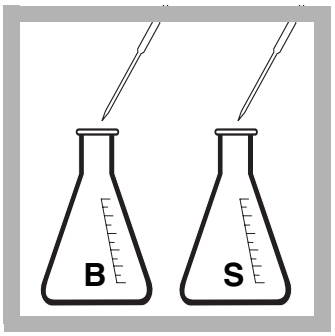
Measure 100 mL of sample into a 500-mL Erlenmeyer flask. Label the flask “sample”.



4. Add a 0.2-g spoonful of TitraVer® Hardness Reagent to each flask. Swirl to mix.

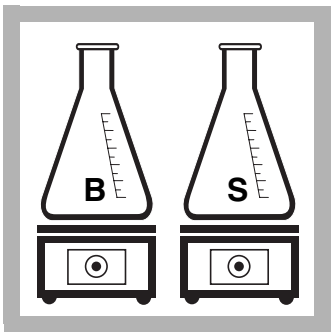


5. Add a 0.05-g spoonful of diaminobenzidene tetrahydrochloride to each flask. Swirl to mix.



6. If you have not distilled the sample, add 5.0 mL of Buffer Solution, sulfate type, pH 2.0 to each flask. Swirl to mix.

If the sample has been distilled, adjust the pH of the distillate to pH 2.7 ± 0.2 using 5 N Sodium Hydroxide Standard Solution. Adjust the blank to pH 2.7 ± 0.2 using 5.25 N Sulfuric Acid Standard Solution.



7. Heat each flask on a hot plate. Bring the contents to a gentle boil.

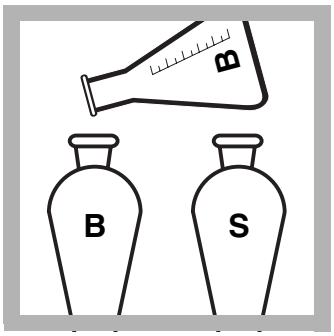
8. Press **TIMER>OK**.

A five-minute reaction period will begin. Continue to boil the contents gently during this time period.

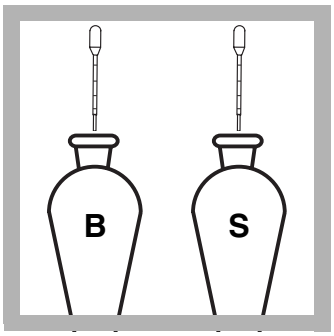
A yellow color will develop if selenium is present.

9. When the timer expires, remove both flasks. Cool to room temperature using a water bath.

Do not boil more than one minute after the timer expires.



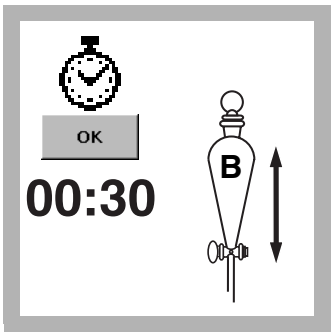
10. Transfer the contents of each flask to separate 250-mL separatory funnels. Label the funnels “blank” and “sample”.



11. Add 2.0 mL of 12 N Potassium Hydroxide Standard Solution to each funnel using a calibrated 1.0-mL plastic dropper. Stopper. Shake each funnel to mix.

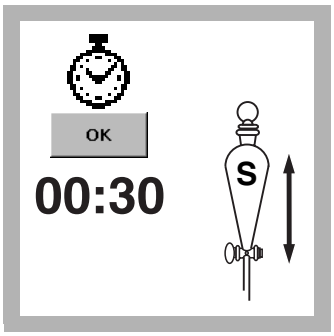
12. Add 30-mL of toluene to each funnel. Stopper. Swirl and invert each funnel, then open the stopcock to vent the funnel. Close the stopcock. Repeat twice with each funnel.

Use toluene only with adequate ventilation.



13. Press **TIMER>OK**.

A 30-second reaction period will begin. During this time, vigorously shake the funnel that contains the blank.

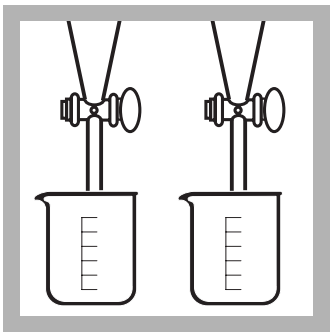


14. Press **TIMER>OK**.

A 30-second reaction period will begin. During this time, vigorously shake the funnel that contains the sample.

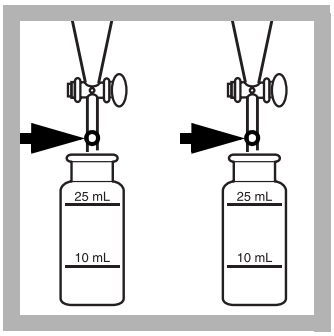
15. Press **TIMER>OK**.

A four-minute reaction period will begin.



16. When the timer expires, drain the lower water layer from each funnel and discard.

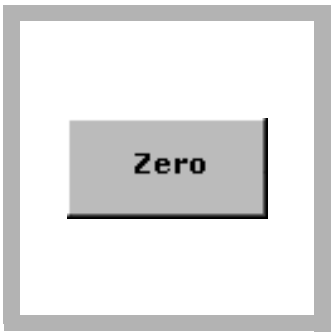
Complete steps 17–20 within five minutes after the timer expires. The developed color is stable, but should be measured as soon as possible.



17. Insert a cotton plug into the delivery tube of each separatory funnel. Slowly drain the toluene into respective sample cells labeled “blank” and “sample”. Stopper the sample cells.

Filtering the toluene through dry, absorbent cotton will remove water or suspended particles.

18. Wipe the blank and insert it into the cell holder with the fill line facing the user.



19. Press **ZERO**.

The display will show:

0.00 mg/L Se

20. Wipe the prepared sample and insert it into the cell holder with the fill line facing the user. Results are in mg/L Se.

Interferences

Table 1 Interfering Substances and Levels

Interfering Substance	Interference Levels and Treatments
Ferric iron	Up to 2.5 mg/L. Distill sample to eliminate interference.
Manganese	Will not interfere.
Strong oxidizing agents (i.e., iodine, bromine, or chlorine)	Can react with the indicator to give low results. Distill sample to eliminate interference.

Note: *There are no positive inorganic interferences with this method.*

Sample Collection, Storage, and Preservation

Collect samples in clean glass or plastic containers. Adjust the pH to 2 or less with Nitric Acid* (about 1.5 mL per liter). Preserved samples can be stored for up to six months at room temperature. Correct the test result for volume additions.

* See Optional Reagents and Apparatus on page 8.

Distillation

CAUTION
Always perform this procedure under a fume hood!

This distillation involves the use of a strong acid and oxidizer at high temperatures. To avoid personal injury, observe all laboratory safety precautions when operating the distilling apparatus.

1. Measure 500 mL of sample into a 1000-mL beaker.
2. Add 1 mL of Methyl Orange Indicator Solution. Stir with a glass rod.
3. Use a dropper to add 0.1 N Hydrochloric Acid Standard Solution dropwise until the solution becomes pink. Then add an additional 2 mL.
4. Use a pipet to add 5.0 mL Calcium Chloride Solution. Mix well.
5. Use a dropper to add 1-g/L Potassium Permanganate Standard Solution drop-wise until the solution is purple.
6. Place the beaker on a hot plate. Evaporate the solution to approximately 250 mL. Periodically add 1-g/L Potassium Permanganate Solution to keep the solution purple.
7. Any precipitate formed at this step is manganese dioxide, and may be ignored.
8. Cool the solution. While cooling, set up the distillation apparatus for the general purpose distillation as shown in the distillation manual.
9. Pour the treated sample solution into the distillation flask. Add a stirring bar to the flask.
10. Pipet 5.0 mL of 0.1 N Sodium Hydroxide Standard Solution into the flask. Turn the stirrer power switch to ON. Set the stir control to 5.
11. Turn on the water and adjust so a constant flow is maintained through the condenser. Set the heat control to 10.
12. When only a few milliliters are left in the distillation flask, turn the power switch off. The distillate in the Erlenmeyer flask may be discarded.

CAUTION
Perform step 13 under a fume hood.

13. When the flask has cooled, add 50 mL of 19.2 N Sulfuric Acid Standard Solution to the flask. Add the contents of one Potassium Bromide Powder Pillow to the flask.
14. Fill a 250-mL beaker to the 75-mL mark with deionized water. Place it under the drip tube. Elevate the beaker with a laboratory jack so the tube extends below the level of the water.
15. Add 1.0 mL of 30% hydrogen peroxide solution to the flask. Turn the stir control to 5 and the heat control to 10. Cap the distillation flask.
16. Heat the distillation flask until the yellow color is gone from the complete distillation apparatus, including the J-tube and condenser. Remove the beaker from under the drip tube.
17. Turn off the heater switch. When the J-tube and condenser have cooled, rinse them with deionized water. Add the washings to the 250-mL beaker. Total volume in the beaker should be approximately 100 mL.
18. Add the Phenol Solution drop-wise to the distilled sample to discharge the bromine color (a white precipitate of tribromophenol will form).

19. Allow the precipitate to settle. Using a dropper, collect about 5 mL of the clear, colorless distillate and transfer to a test tube.

20. Test the solution for completeness of precipitation by adding 2 drops of Phenol Solution. If the solution becomes cloudy or white precipitate forms, residual bromine is still present (proceed to next step). If no cloudiness occurs, the sample is ready for analysis.

21. Transfer the 5-mL aliquot back to the beaker and continue to add Phenol Solution until no turbidity is formed in subsequent 5-mL aliquots.

22. Transfer the entire sample into a 500-mL volumetric flask. Rinse the beaker with deionized water and add to the flask.

23. Dilute to volume with deionized water, stopper and mix well. The distillate is now ready for analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.

2. Press **OPTIONS>MORE**. Press **STANDARD ADDITIONS**. A summary of the standard additions procedure will appear.

3. Press **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Press **EDIT** to change these values. After values are accepted, the unspiked sample reading will appear in the top row.

4. Prepare a 100 mg/L selenium standard solution by pipetting 10 mL of 1000 mg/L Selenium Standard Solution into a 100 mL volumetric flask, and diluting to volume with demineralized water.

5. Prepare three sample spikes. Fill three mixing cylinders with 100 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.

6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by pressing **READ**. Each addition should reflect approximately 100% recovery.

7. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Press **VIEW: FIT**, then select **IDEAL LINE** and press **OK** to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

Standard Solution Method

Prepare a 0.5-mg/L Se standard solution as follows:

1. Prepare a 100 mg/L selenium standard solution by pipetting 10 mL of 1000 mg/L Selenium Standard Solution into a 100 mL volumetric flask, and diluting to volume with demineralized water. Pipet 1.00 mL of this 100 mg/L standard into a 200 mL volumetric flask. Dilute to volume with deionized water. Transfer 100 mL of the standard into a 500-mL Erlenmeyer flask. Perform the test as described above.

2. To adjust the calibration curve using the reading obtained with the 0.5-mg/L standard solution, press **OPTIONS>MORE** on the current program menu. Press **STANDARD ADJUST**.

3. Press **ON**. Press **ADJUST** to accept the displayed concentration. If an alternate concentration is used, press the number in the box to enter the actual concentration, then press **OK**. Press **ADJUST**.

Method Performance

Precision

Standard: 0.50 mg/L Se

Program	95% Confidence Limits of Distribution
640	0.47–0.53 mg/L Se

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.01 mg/L Se

Summary of Method

An EDTA masking agent is added to the sample to remove interferences such as iron prior to the test. The addition of a sulfate buffer adjusts the sample to the optimum pH of 1 to 2. Under these conditions, diaminobenzidine reacts with all selenium present as selenite (Se^{4+}) to give a yellow-colored piazselenol complex which is extracted and the color intensity measured colorimetrically. Selenium present as Se^{2+} and Se^{6+} is not detected unless the sample is distilled. Test results are measured at 420 nm.

Consumables and Replacement Items

Required Reagents

Description	Quantity/Test	Unit	Cat. No.
Selenium Reagent Set (100 tests), includes:			22442-00
(1) Buffer Solution, sulfate type, pH 2.0	10 mL	500 mL	452-49
(1) Diaminobenzidine, tetrahydrochloride	0.1 g	5 g	7062-22
(2) Potassium Hydroxide Standard Solution, 12 N	4 mL	100 mL	230-32
(1) TitraVer® Hardness Reagent, ACS	0.4 g	100 g	204-26
(1) Toluene, ACS	60 mL	4 L	14470-17
Water, deionized	100 mL	4 L	272-56

Required Apparatus

Description	Quantity/Test	Unit	Cat. No.
Cotton Balls, absorbent	1	100/pkg	2572-01
Cylinder, graduated, 50-mL	1	each	508-41
Cylinder, graduated, 100-mL	1	each	508-42
Dropper, 0.5 & 1.0 mL marks, glass	1	5/pkg	14197-05
Dropper, 0.5 & 1.0 mL marks, plastic	1	20/pkg	21247-20
Flask, Erlenmeyer, 500-mL	2	each	505-49
Funnel, separatory, 250-mL	2	each	520-46
Hot Plate, 4-inch diameter, 120 VAC	1	each	12067-01
OR			
Hot Plate, 4-inch diameter, 240 VAC	1	each	12067-02
Pipet, volumetric, 5-mL	1	each	14515-37
Pipet filler, safety bulb	1	each	14651-00

Required Apparatus (continued)

Description	Quantity/Test	Unit	Cat. No.
Ring, support, (3-inch) 83-mm	1	each	580-00
Sample Cells, 1-inch square, 25 mL with stopper, matched pair	2	2/pkg	26126-02
Spoon, measuring, 0.05-g	1	each	492-00
Spoon, measuring, 0.2-g	1	each	638-00
Support, ring stand, (5 x 8 inch) 127 x 203 mm	1	each	563-00

Distillation Reagents and Apparatus

Description	Unit	Cat. No.
Calcium Chloride Solution	1000 mL	428-53
Hydrochloric Acid Standard Solution, 0.1 N	1000 mL	14812-53
Hydrogen Peroxide, 30%, ACS	473 mL	144-11
Methyl Orange Indicator Solution, (0.50-g/L)	500 mL	148-49
Phenol Solution, 30-g/L	29 mL	2112-20
Potassium Permanganate Standard Solution, 1-g/L	100 mL	14164-42
Sodium Hydroxide Standard Solution, 0.100 N	1000 mL	191-53
Sulfuric Acid Standard Solution, 19.2 N	500 mL	2038-49
Distillation Apparatus Set, general purpose	each	22653-00
Distillation Apparatus Heater, 115 VAC	each	22744-00
Distillation Apparatus Heater, 230 VAC	each	22744-02

Recommended Standards

Description	Unit	Cat. No.
Selenium Standard Solution, 1000-mg/L	100 mL	22407-42

Optional Reagents and Apparatus

Description	Cat. No.
Acetone	14429-49
Nitric Acid	152-49
Sodium Hydroxide, 5.0 N	2450-32
Sulfuric Acid, 5.25 N	2449-32

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Updated April 05 Edition 1

Sulfate

★Method 8051 — SulfaVer 4 Method[1]

Powder Pillows or AccuVac® Ampuls — (2 to 70 mg/L)

Scope and Application: For water, wastewater, and seawater; USEPA accepted for reporting wastewater analyses

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater*. Procedure is equivalent to USEPA method 375.4 for wastewater.

Test Preparation

Before starting the test:

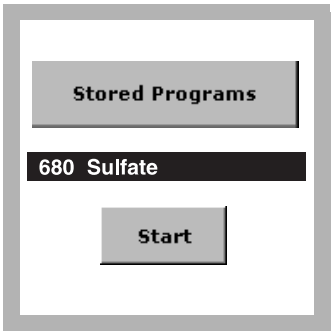
Adjust the standard curve for each new lot of reagent (Standard Solutions on page 4).
For best results, perform a new calibration for each lot of reagent (Calibration Standard Preparation on page 5).
For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
Filter highly colored or turbid samples using filter paper[1] and a funnel[1]. Use this sample in step 3 and 6. Undissolved reagent powder that has settled does not affect accuracy.
SulfaVer® 4 contains barium chloride. The final solution will contain barium chloride (D005) at a concentration regulated as a hazardous waste by the Federal RCRA. Refer to a current MSDS for safe handling and disposal instructions.

Collect the following items:	**Quantity**
Powder Pillow Test:	
SulfaVer® 4 Reagent Powder Pillows	1
Sample Cells, 1-inch square, 10-mL	2
AccuVac Test:	
SulfaVer® 4 Reagent AccuVac® Ampuls	1
Beaker, 50-mL	1
Sample Cell, 10-mL round, with cap	1
Stopper for 18 mm Tube	1

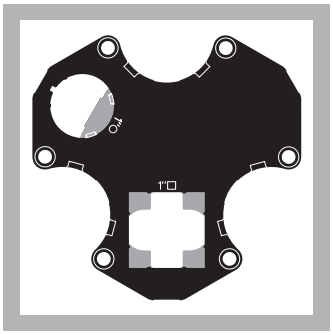
Note: *Reorder information for consumables and replacement items is on page 6.*

[1] See Optional Reagents and Apparatus on page 6.

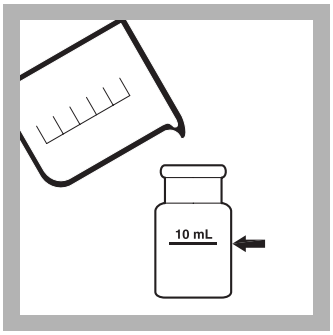
Powder Pillows Method 8051



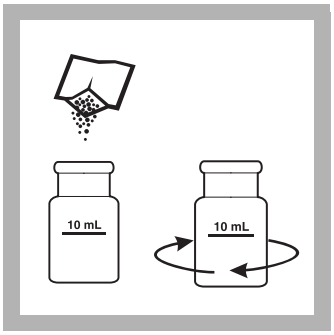
1. Select the test.



2. Insert the Multi-cell Adapter with the 1-inch square cell holder facing the user.



3. Fill a square sample cell with 10 mL of sample.

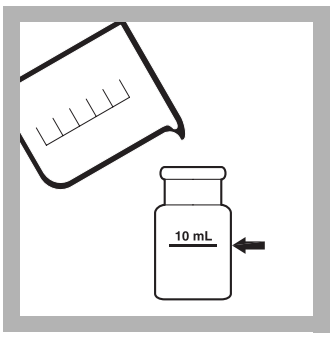


4. Prepared Sample: Add the contents of one SulfaVer 4 Reagent Powder Pillow to the sample cell. Swirl vigorously to dissolve powder.

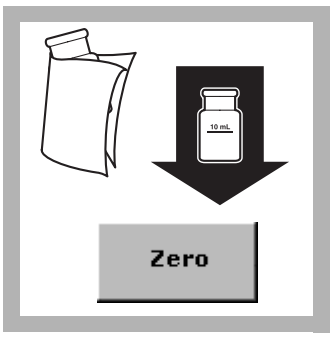
White turbidity will form if sulfate is present.

5. Press **TIMER>OK**.

A five-minute reaction period will begin. Do not disturb the cell during this time.



6. Blank Preparation: Fill a second square sample cell with 10 mL of sample.



7. When the timer expires, insert the blank into the cell holder with the fill line facing the user.

Press **ZERO**.

The display will show:

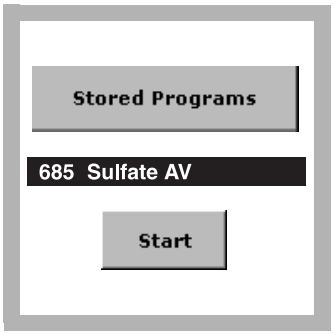
0 mg/L SO_4^{2-}

8. Within five minutes after the timer expires, insert the prepared sample into the cell holder with the fill line facing the user.

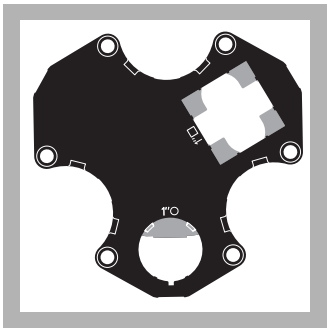
Results are in mg/L SO_4^{2-}.

Clean sample cells with a soap and brush.

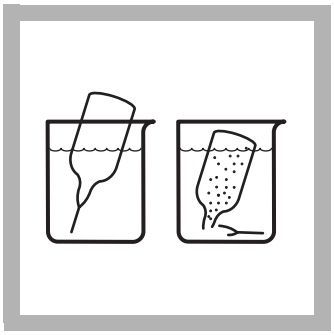
AccuVac Ampul — Method 8051



1. Select the test.

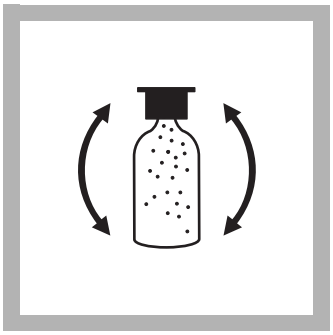


2. Insert the Multi-cell Adapter with the 1-inch round cell holder facing the user.



3. Prepared Sample: Collect at least 40 mL of sample in a 50-mL beaker.

Fill a SulfaVer 4 Sulfate AccuVac Ampul with sample. Keep the tip immersed until the ampule fills completely.

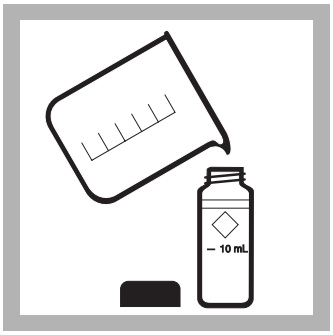


4. Quickly invert the ampule several times to mix.

White turbidity will form if sulfate is present.

5. Press **TIMER>OK**.

A five-minute reaction period will begin. Do not disturb the cell during this time.



6. Fill a clean sample cell with 10 mL of sample.

7. When the timer expires, insert the blank into the cell holder.

Press **ZERO**.

The display will show:

0 mg/L SO_4^{2-}

8. Within five minutes after the timer expires, insert the ampule into the cell holder.

Results are in mg/L SO_4^{2-}.

Interferences

Table 1 Interfering Substances and Levels

Interfering Substance	Interference Levels and Treatments
Calcium	Greater than 20,000 mg/L as $CaCO_3$
Chloride	Greater than 40,000 mg/L as Cl^-
Magnesium	Greater than 10,000 mg/L as $CaCO_3$
Silica	Greater than 500 mg/L as SiO_2

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Samples may be stored up to 7 days by cooling to 4 °C (39 °F) or lower. Warm to room temperature before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.

2. Press **OPTIONS>MORE**. Press **STANDARD ADDITIONS**. A summary of the standard additions procedure will appear.

3. Press **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Press **EDIT** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.

4. Snap the neck off a Sulfate Ampule Standard, 2500-mg/L sulfate.

5. Prepare three sample spikes. Fill three mixing cylinders* with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.

6. Transfer 10 mL of each sample spike to a clean sample cell and analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by pressing **READ**. Each addition should reflect approximately 100% recovery.

Note: *For AccuVac® Ampuls, fill three Mixing Cylinders* with 50 mL of sample and spike with 0.2 mL, 0.4 mL, and 0.6 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL Beakers†. Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by pressing* ***READ****. Each addition should reflect approximately 100% recovery.*

7. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points, accounting for the matrix interferences. Press **IDEAL LINE** to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

Standard Solutions

Prepare a 70-mg/L sulfate standard solution as follows:

1. Using Class A glassware, Pipet 7 mL of Sulfate Standard Solution, 1000-mg/L, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the SulfaVer procedure as described above.

2. To adjust the calibration curve using the reading obtained with the standard solution, press **OPTIONS>MORE** on the current program menu. Press **STANDARD ADJUST**.

3. Press **ON**. Press **ADJUST** to accept the displayed concentration. If an alternate concentration is used, press the number in the box to enter the actual concentration, then press **OK**. Press **ADJUST**.

* See Optional Reagents and Apparatus on page 6.
†See Required Apparatus (AccuVac) on page 6.

Calibration Standard Preparation
To perform a sulfate calibration using the SulfaVer method, use Class A glassware to prepare calibration standards containing 10, 20, 30, 40, 50, 60, and 70 mg/L SO_4^{2-} as follows:

1. Into seven different 100-mL Class A volumetric flasks, pipet 1, 2, 3, 4, 5, 6, and 7 mL of the 1000-mg/L Sulfate Standard Solution.

2. Dilute to the mark with deionized water. Mix thoroughly.

3. Using the SulfaVer method and the calibration procedure described in the User Programs section of the user manual, generate a calibration curve from the calibration standards prepared above.

Method Performance

Precision
Standard: 40 mg/L SO_4^{2-}

Program	95% Confidence Limits of Distribution
680	30–50 mg/L SO_4^{2-}
685	32–48 mg/L SO_4^{2-}

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
680	Entire range	0.010	0.4 mg/L SO_4^{2-}
685	30 mg/L	0.010	0.7 mg/L SO_4^{2-}

Summary of Method

Sulfate ions in the sample react with barium in the SulfaVer 4 and form a precipitate of barium sulfate. The amount of turbidity formed is proportional to the sulfate concentration. Test results are measured at 450 nm.

Consumables and Replacement Items

Required Reagents

Description	Quantity/Test	Unit	Cat. No.
SulfaVer® 4 Reagent Powder Pillows	1	100/pkg	21067-69
OR			
SulfaVer® 4 Sulfate Reagent AccuVac® Ampuls	1	25/pkg	25090-25

Required Apparatus (Powder Pillows)

Description	Quantity/Test	Unit	Cat. No.
Sample Cells, 1-inch square, 10 mL, matched pair	2	2/pkg	24954-02

Required Apparatus (AccuVac)

Description	Quantity/Test	Unit	Cat. No.
Beaker, 50-mL	1	each	500-41H
Sample Cell, 10-mL, with cap	1	6/pkg	24276-06
Stopper for 18 mm Tube	1/test	6/pkg	1731-06

Recommended Standards

Description	Unit	Cat. No.
Sulfate Standard Solution, 1000-mg/L	500 mL	21757-49
Sulfate Standard Solution, 2500-mg/L, 10-mL Ampules	16/pkg	14252-10
Standard, Drinking Water, Mixed Parameter, Inorganic for F^-, NO_3, PO_4, SO_4	500 mL	28330-49

Optional Reagents and Apparatus

Description	Unit	Cat. No.
Cylinder, mixing, 25-mL	each	1896-40
Cylinder, mixing, 50 mL	each	1896-41
Stopper for 18 mm Tube	25/pkg	

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Updated April 05 Edition 1

Trihalomethanes

Method 10132
Water Bath Method

THM Plus™ Method
(10 to 600 ppb as Chloroform)

Scope and Application: For screening THMs in drinking water samples and Formation Potential tests.

Test Preparation

Before starting the test:

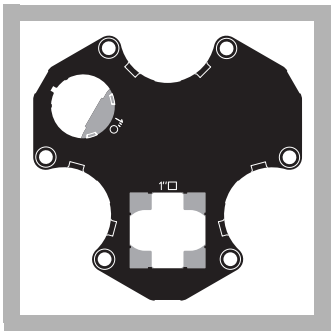
If analyzing more than four samples, use 450 mL of water in the water bath.
THM Plus Reagent 2 **must** be at room temperature before use.
A Repipet Jr. may be used in place of the TenSette® Pipet.
Trihalomethane compounds are extremely volatile. Immediately cap sample cells after filling with sample.
Reagent blank is stable for 1–2 hours and need not be prepared for each test.

Collect the following items:	**Quantity**
THM Plus Reagent Set	varies
Beaker, 600-mL	1
Cell Holder Assembly, TTHM	1
Evaporating Dish, 125 mm x 65 mm	1
Hot Plate, 7 x 7 inch	1
Pipet, TenSette®, 0.1–1.0 mL and tips	1
Pipet, TenSette®, 1–10 mL and tips	1
Sample Cells, 10 mL, with caps.	2
Sample Cells, 10-mL, 1-inch square, matched pair	2
Wipers, disposable, KimWipes®	varies

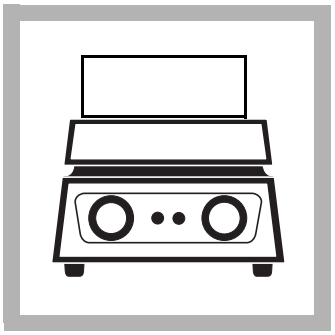
Note: *Reorder information for consumables and replacement items is on page 8.*

Method 10132

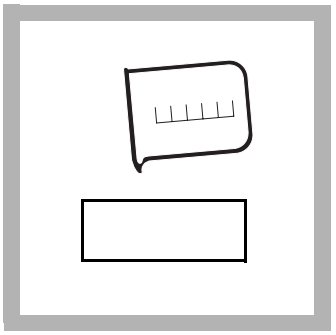
1. Select the test.



2. Insert the Multi-cell Adapter with the 1-inch square cell holder facing the user.



3. Prepare a hot water bath by adding 500 mL of water to an evaporating dish. Put the dish on a hot plate and turn the heater on high.

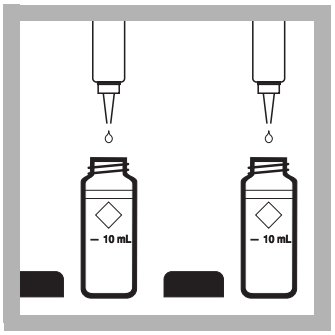


4. Prepare a cooling bath by adding 500 mL of cold (18–25 °C) tap water to a second evaporating dish. Maintain the temperature in this range.

Important Note: *Perform steps 5–10 rapidly to avoid loss of THMs from the sample. When testing more than one sample, complete steps 5–10 for one sample before going on to another. If dispensing sample with a pipette, the pipette must dispense quickly without causing aeration or back pressure.*

5. Prepared Sample: Fill one round sample cell to the 10-mL mark with sample. Cap and label as “sample”.

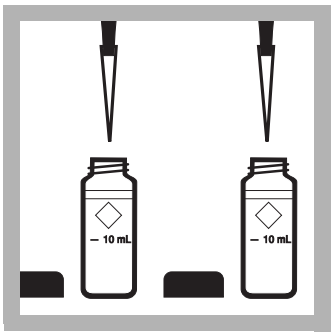
6. Blank Preparation: Fill the second sample cell with deionized water. Cap and label as “blank”.



7. Add three drops of THM Plus Reagent 1 to each cell.

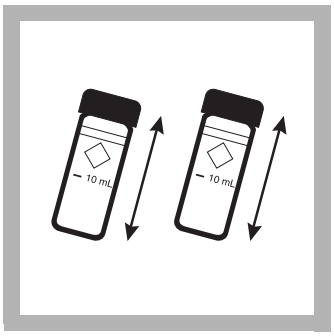
8. Cap tightly and mix gently by swirling each cell three times.

Vigorous shaking can cause loss of THMs into the sample cell headspace.



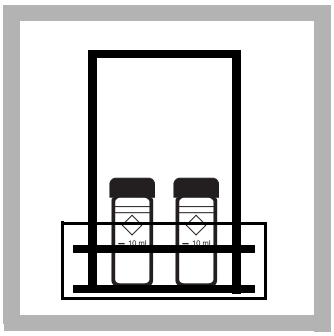
9. Use a TenSette® Pipet to add 3 mL of THM Plus Reagent 2 to each cell. Avoid excess agitation of the sample when dispensing the reagent.

The reagent is viscous and a small amount may remain on the tip after dispensing. This will not affect the results.

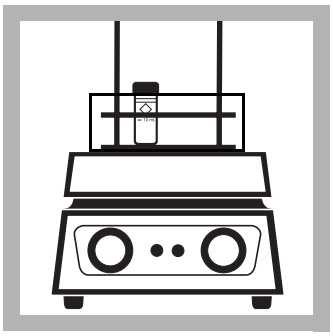


10. Cap tightly and mix by shaking.

Thorough mixing ensures that all of the THM goes into the liquid and does not accumulate in the air above the sample.



11. Place the sample cells in the cell holder assembly.

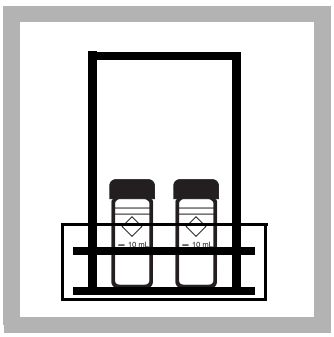


12. Place the assembly in the hot water bath when the water is boiling rapidly.

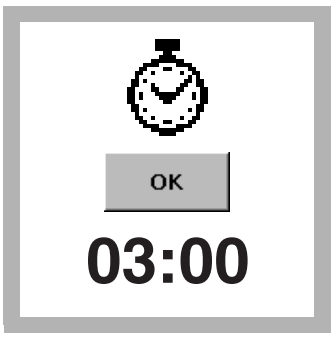
Do not allow the water to rise above the white “diamond” near the top of the sample cells.

13. Press **TIMER>OK**.

A five-minute reaction period will begin.



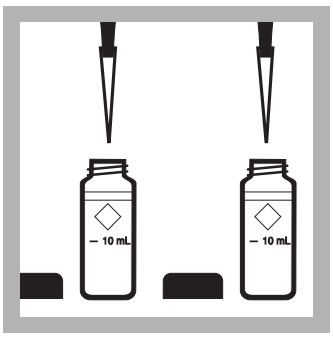
14. When the timer expires, remove the assembly and sample cells from the hot water bath. Place in the cooling bath.



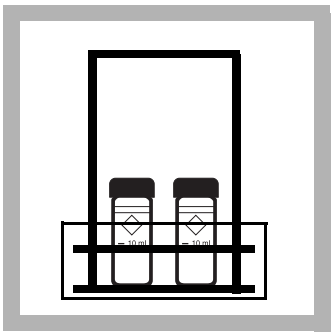
15. Press **TIMER>OK**.

A three-minute cooling period will begin.

When the timer expires, remove the cells from the cooling bath.



16. Use a TenSette Pipet to add 1 mL of THM Plus Reagent 3 to each cell. The sample and blank will become warm.



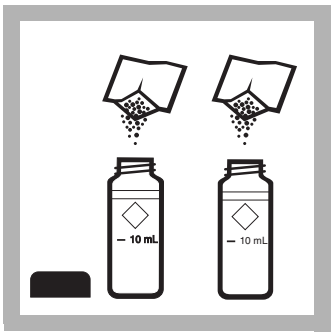
17. Replace the cooling water with fresh, cold tap water. Place the assembly that contains the sample and blank cells into the cooling bath.

18. Press **TIMER>OK**.

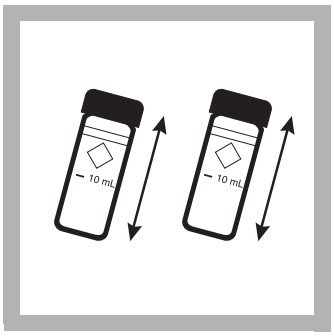
A second three-minute cooling period will begin.

After the timer expires, remove the cells from the cooling bath.

The temperature of the sample should be 15–25 °C.



19. Add the contents of one THM Plus Reagent 4 Powder Pillow to the sample cell and one to the blank.

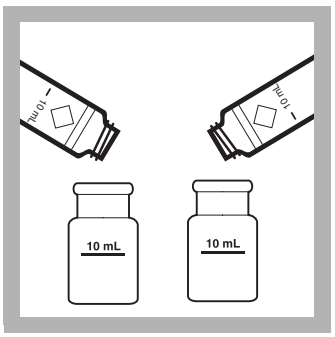


20. Cap each cell tightly and mix by shaking until all the powder dissolves.

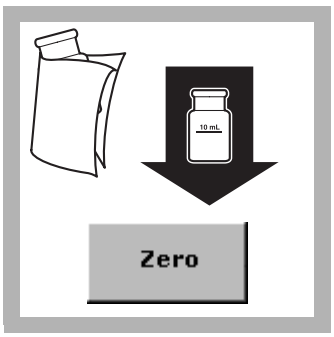
The powder dissolves slowly. Intermittent shaking during the first five minutes of the color development period will help dissolve the reagent powder.

21. Press **TIMER>OK**.

A 15-minute development time will begin.



22. After the timer expires, pour the prepared sample and prepared blank into two square sample cells.

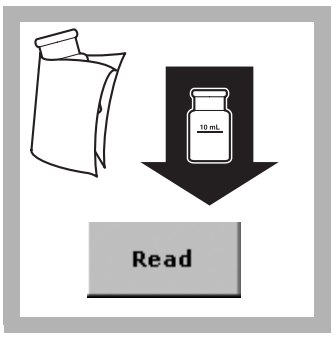


23. Insert the blank into the cell holder with the fill line facing the user. Close the cover.

Press **ZERO**.

The display will show:

0 ppb $CHCl_3$



24. Insert the prepared sample into the cell holder with the fill line facing the user. Close the cover.

Press **READ**.

Results are in ppb chloroform.

“Underrange” will appear in the display if results are below the Estimated Detection Limit.

Interferences

The substances in Table 1 have been tested and found not to interfere up to the indicated levels (in ppm):

Table 1 Interferences That Have No Effect Up to the Maximum Level Tested

Interfering Substance	Interference Levels and Treatments
Chlorine	<10 ppm
Copper	<1000 ppm
Hardness, Ca	<1000 ppm as $CaCO_3$ May have some turbidity until Reagent 3 is added
Hardness, Mg	<4000 ppm as $CaCO_3$ May have some turbidity until Reagent 3 is added
Iron	<10 ppm
Lead	<2 ppm
Mercury	<10 ppm
Monochloramine	<20 ppm
Nickel	<10ppm
Sodium Bisulfite	<100 ppm
EDTA	Interferes negatively at all levels

Table 2 Additional Disinfection By-Products That React

Compound	Effect
1,1,1-trichloro-2-propanone	Interferes positively
1,1,1-tricholoacetonitrile	Interferes positively
Chloral hydrate	Interferes positively
Dibromochloroacetic acid	Interferes positively
Dichlorobromoacetic acid	Interferes positively
Tribromoacetic acid	Interferes positively
Trichloroacetic acid	Interferes positively

Sampling and Storage

Collect samples in 40-mL glass bottles sealed with Teflon®-lined septa caps. Fill the bottles slowly to overflowing so that no air is included with the sample. Seal the bottles tightly and invert to check that no air has been trapped.

Because trihalomethane compounds (THMs) are extremely volatile, immediate analysis yields the greatest accuracy. If the samples cannot be analyzed immediately, cool samples to 4 °C. This will slow the formation of any additional THM compounds in chlorinated samples. Store the samples at 4 °C in an atmosphere free of organic vapors. Samples should not be held more than 14 days. Allow the samples to equilibrate to 15–25 °C before analyzing.

Accuracy Check

Standard Additions Method (Sample Spike)
Prepare the standard additions sample at the same time as the unspiked water sample.

1. Snap the neck off a THM Standard Ampule, 10 ppm as chloroform.

2. Using a Wiretrol™ Pipet, add 0.050 mL of the standard to 10 mL of water sample. Immerse the tip of the pipet below the surface of the water sample and dispense the aliquot of chloroform standard.

3. Cap the sample cell immediately and swirl three times to mix. Prepare the sample and the spiked sample according to the procedure steps 7–24.

4. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify that the units displayed are in ppb.

5. Press **OPTIONS>MORE**. Press **STANDARD ADDITIONS**. A summary of the standard additions procedure will appear.

6. Press **OK** to accept the default values for standard concentration, sample volume, and spike volume. Press **EDIT** to change these values. After values are accepted, the unspiked sample reading will appear in the top row

7. Insert the prepared spiked sample into the cell holder. Press **READ**.

8. After completing the sequence, press **GRAPH** to view the best-fit line through the standard additions data points. Press **IDEAL LINE** to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery. The addition should reflect 80–120% recovery.

CAUTION
Chloroform is extremely volatile! Do not shake it when mixing.

Standard Solutions Method
Prepare a 99 ppb chloroform standard by pipetting 10.0 mL of organic-free water into a sample cell. Snap the neck off a THM Standard Ampule, 10 ppm as chloroform. Using a Wiretrol Pipette, transfer 0.100 mL of the chloroform standard into the organic-free water. Immerse the end of the pipet tip under the water to dispense the chloroform. Cap the sample cell immediately and swirl three times to mix. Immediately perform steps 7–24 of the procedure. Do not make up the standard in advance. Use the standard immediately upon preparation.

Method Performance

Precision
Tap Water Sample: 66 ppb $CHCl_3$

Program	95% Confidence Limits
725	53–79 ppm $CHCl_3$

Sensitivity

Portion of Curve	Δ ABS	Δ Concentration
Entire range	0.010	19 ppb $CHCl_3$

Summary of Method

The THM Plus method reacts with the trihalogenated disinfection by-products formed as the result of the disinfection of drinking water with chlorine in the presence of naturally occurring organic materials. These disinfection by-products (DBPs) may be produced in the treatment plant or the distribution system as long as the water is in contact with free chlorine residual. The formation of the DBPs is influenced by chlorine contact time, chlorine dose and residual, temperature, pH, precursor concentration, and bromide concentration.

The predominant DBPs formed by the chlorination of drinking water are the trihalomethanes or THMs. The four trihalogenated compounds that form are chloroform, bromoform, dichlorobromomethane, and dibromochloromethane. These four compounds comprise the Total Trihalomethanes (TTHMs) group which is regulated under the Safe Drinking Water Act. The combined concentration of the TTHMs, is regulated to be 80 ppb or less in drinking water samples. Other DBPs that may be present and react under the conditions of the THM Plus method are listed in Interferences.

In the THM Plus method, THM compounds present in the sample react with N, N,-diethylnicotinamide under heated alkaline conditions to form a dialdehyde intermediate. The sample is then cooled and acidified to pH 2.5. The dialdehyde intermediate formed is then reacted with 7-amino-1,3 napthalene disulfonic acid to form a colored Schiff base. The color formed is directly proportional to the total amount of THM compounds present in the sample. Test results are measured at 515 nm and reported as ppb chloroform.

Consumables and Replacement Items

Required Apparatus

Description	Quantity/Test	Unit	Cat. No.
Reagent Set (50 tests[1]), includes:			27908-00
THM Plus™ Reagent 1	6 drops	15 mL	27539-29
THM Plus™ Reagent 2	6 mL	330 mL	27540-48
THM Plus™ Reagent 3	2 mL	110 mL	27541-42
THM Plus™ Reagent 4	2 pillows	100 pillows	27566-99

[1] Fifty tests equals 25 samples and 25 individual blanks. Additional tests can be obtained when multiple samples are run using a single blank.

Required Apparatus

Description	Quantity/Test	Unit	Cat. No.
Beaker, 600-mL	1	each	500-52
Cell Holder Assembly, TTHM	1	each	47880-00
Evaporating Dish, 125 mm x 65 mm	1	each	27647-00
Hot Plate, 7 x 7 in., 120 VAC, digital	1	each	28816-00
Hot Plate, 7 x 7 in., 240 VAC, digital	1	each	28816-02
Pipet, TenSette®, 0.1–1.0 mL	1	each	19700-01
Pipet Tips for TenSette Pipet 19700-01	varies	50/pkg	21856-96
Pipet, TenSette®, 1–10 mL	1	each	19700-10
Pipet Tips, for TenSette Pipet 19700-10	varies	50/pkg	25589-96
Sample Cells, 10 mL, with caps.	2	6/box	24276-06
Sample Cells, 1-inch square, 10 mL, matched pair	2	2/pkg	24954-02
Wipers, disposable, KimWipes®	varies	280/pkg	20970-00

Recommended Standards

Description	Unit	Cat. No.
Chloroform, 10-ppm ampule	each	27567-07
Water, Reagent, Organic-free	500 mL	26415-49

Recommended Apparatus

Description	Unit	Cat. No.
Flask, volumetric, 100 mL, class A	.each	14574-42
Pipet, filler, safety bulb	each	14651-00
Pipet, volumetric, class A, 10 mL	each	14515-38
Pipettes, Wiretrol™, 50–100 µL	250/pkg	25689-05
Repipet Jr., 1-mL	each	21113-02
Vials, glass, 40-mL, with Septa cap	5/pkg	27940-05

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Updated April 05 Edition 1

Hardness, Total

Method 8213

Digital Titrator with EDTA Method

Digital Titrator

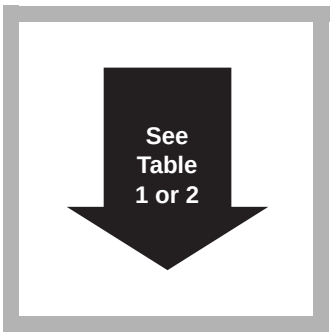
(10 to 4000 mg/L as $CaCO_3$)

Scope and Application: For water, wastewater, and seawater

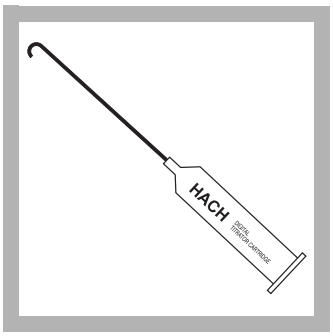
Tips and Techniques

- One German degree of hardness (G.d.h.) = 17.9 mg/L hardness as $CaCO_3$
- You can substitute a 0.1-g scoop of ManVer® 2 Hardness Indicator Powder or four drops of ManVer Hardness Indicator Solution for the ManVer 2 Hardness Indicator Powder Pillow.
- For added convenience when stirring, use the TitraStir apparatus (Cat. No. 19400-00, -10).
- mg/L Total Hardness as Ca = mg/L Total Hardness as $CaCO_3$ x 0.40
- mg/L Total Hardness as $CaCO_3$ = mg/L Ca as $CaCO_3$ + mg/L Mg as $CaCO_3$
- The magnesium concentration may be determined by subtracting the results of the calcium determination from the total hardness determination.

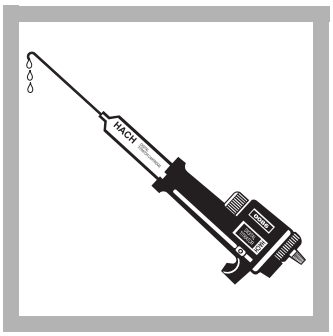
Digital Titration Method 8213



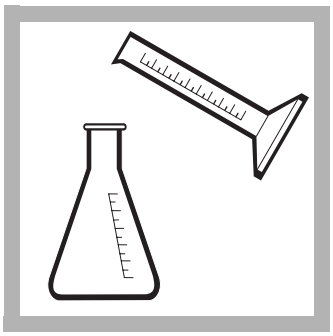
1. Select a sample size and an EDTA Titration Cartridge that corresponds to the expected calcium as calcium carbonate ($CaCO_3$) concentration. Use *Table 1* for concentrations in mg/L and *Table 2* for concentrations in German degrees of hardness.



2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator body.

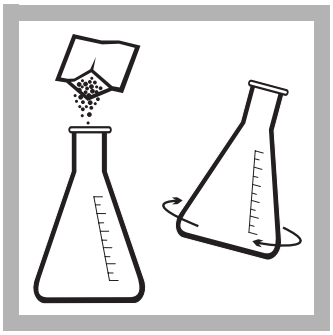


3. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

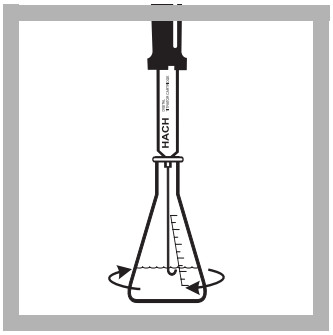


4. Use a graduated cylinder or pipet to measure the sample volume from *Table 1* or *Table 2*. Transfer the sample into a clean 250-mL Erlenmeyer flask. Dilute to about the 100-mL mark with deionized water, if necessary.

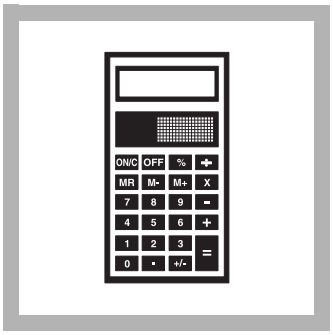
5. Add 2 mL of Buffer Solution, Hardness 1 and swirl to mix.



6. Add the contents of one ManVer 2 Hardness Indicator Powder Pillow and swirl to mix.



7. Place the delivery tube tip into the solution and swirl the flask while titrating with EDTA from red to blue. Record the number of digits required.



8. Calculate the sample concentration using one of the formulas below:

Total Digits Required x Digit Multiplier from *Table 1* = mg/L Total Hardness as $CaCO_3$

Total Digits Required x Digit Multiplier from *Table 2* = G.d.h.

Table 1

Range (mg/L as $CaCO_3$)	Sample Volume (mL)	Titration Cartridge (M EDTA)	Catalog Number	Digit Multiplier
10–40	100	0.0800	14364-01	0.1
40–160	25	0.0800	14364-01	0.4
100–400	100	0.800	14399-01	1.0
200–800	50	0.800	14399-01	2.0
500–2000	20	0.800	14399-01	5.0
1000–4000	10	0.800	14399-01	10.0

Table 2

Range (G.d.h.)	Sample Volume (mL)	Titration Cartridge (M EDTA)	Catalog Number	Digit Multiplier
1–4	100	0.1428	14960-01	0.01
4–16	25	0.1428	14960-01	0.04
10–40	50	0.714	14959-01	0.1
25–100	20	0.714	14959-01	0.25
>100	10	0.714	14959-01	0.5

Interferences

WARNING:
Potassium cyanide is toxic. Always add it after the potassium hydroxide. Excess potassium cyanide does not affect results. Dispose of all cyanide wastes by adding an excess of strongly alkaline sodium hypochlorite solution (bleach) with stirring. Use good ventilation. Allow to stand for 24 hours before disposal.

Some transition and heavy metals complex the indicator and prevent the color change at the end point.

Interfering Substance	Interference Levels and Treatments
Acidity	Does not interfere at 10,000 mg/L (as $CaCO_3$).
Alkalinity	Does not interfere at 10,000 mg/L (as $CaCO_3$).
Aluminum	A 0.5-gram scoop of potassium cyanide* (Cat. No. 767-14) raises the permissible aluminum level to 1 mg/L.
Barium	Titrates directly.
Cobalt	Interferes at all levels and must be absent or masked. A 0.5-gram scoop of potassium cyanide* (Cat. No. 767-14) removes interference from up to 100 mg/L cobalt.
Copper	Interferes at levels of 0.10 and 0.20 mg/L. A 0.5-gram scoop of potassium cyanide* (Cat. No. 767-14) removes interference from up to 100 mg/L copper.
Iron	Does not interfere up to 15 mg/L. Above this level it causes a red-orange to green end point which is sharp and usable up to 30 mg/L iron. Substitute a 0.0800 M CDTA (Cat. No. 14402-01) or 0.800 M CDTA (Cat. No. 14403-01) titration cartridge for the 0.0800 M EDTA or 0.800 M EDTA titration cartridges, respectively, if iron interference is probable.
Manganese	Titrates directly up to 20 mg/L but masks the end point above this level. Adding a 0.1-gram scoop of hydroxylamine hydrochloride monohydrate (Cat. No. 246-14) raises this level to 200 mg/L manganese.
Nickel	Interferes at all levels and must be absent or masked. A 0.5-gram scoop of potassium cyanide* (Cat. No. 767-14) removes interference from up to 100 mg/L nickel.
Orthophosphate	Causes a slow end point.
Polyphosphates	Polyphosphate must be absent for accurate results.
Polyvalent Metal Ions	Although less common than calcium and magnesium, other polyvalent metal ions cause the same hardness effects and will be included in the results.
Sodium Chloride	Saturated sodium chloride solutions do not give a distinct end point, but the titration can be run directly on sea water.
Strontium	Titrates directly.
Zinc	Titrates directly. A 0.5-gram scoop of potassium cyanide* (Cat. No. 767-14) removes interference from up to 100 mg/L zinc.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment: see *Section 3.3 Interferences* on page *50*.

* Metals masked with cyanide will not be included in the hardness result.

Adding the contents of one CDTA Magnesium Salt Powder Pillow removes metal interferences at or below the levels shown in *Table 3*.

Table 3

Metal	CDTA Removes Interference Below This Level
Aluminum	50 mg/L
Cobalt	200 mg/L
Copper	100 mg/L
Iron	100 mg/L
Manganese	200 mg/L
Nickel	400 mg/L
Zinc	300 mg/L

If more than one metal is present at or above the concentrations shown above, an additional CDTA Magnesium Salt Powder Pillow may be required.

Results obtained by this procedure include the hardness contributed by the metals. If the concentration of each metal is known, a correction can be applied to obtain the calcium and magnesium hardness concentration. The hardness (in mg/L as $CaCO_3$) contributed by each mg/L of metal is listed below, and can be subtracted from the total hardness value obtained above to determine the calcium and magnesium hardness. See *Table 4*.

Table 4

Metal	Hardness Contributed by Each mg/L of Metal
Aluminum	3.710
Barium	0.729
Cobalt	1.698
Copper	1.575
Iron	1.792
Manganese	1.822
Nickel	1.705
Strontium	1.142
Zinc	1.531

Sampling and Storage

Collect at least 100 mL of sample in a glass or polyethylene container. Samples may be held up to seven days before analysis if stored at 4 °C and acidified to pH 2 with concentrated nitric acid (Cat. No. 2540-49). Neutralize acidified sample to pH 7 with ammonium hydroxide (Cat. No. 14736-32) before testing.

When significant amounts of preservative are used, a volume correction should be made for the extra acid and base. Divide the total volume (sample + acid + base) by the volume of the sample, and multiply the result by the final test outcome.

Accuracy Check

Standard Additions Method (Sample Spike)

To verify analytical technique use 20 mL of the Calcium Standard Solution, 1000-mg/L as $CaCO_3$. Perform the procedure as described above. This solution will read 1000 mg/L or 55.9 G.d.h.

Perform this accuracy check when interferences are suspected.

1. Snap the neck off a Hardness Voluette Ampule Standard, 10,000-mg/L as $CaCO_3$.
2. Use a TenSette Pipet to add 0.1 mL of standard to the sample titrated in *step 7*. Resume titration back to the same end point. Record the number of digits required.
3. Repeat, using two more additions of 0.1 mL. Titrate to the end point after each addition.
4. Each 0.1 mL addition of standard should require 10 additional digits of 0.800 M titrant, 100 digits of 0.0800 M titrant, 11 digits of 0.714 M, or 56 digits of 0.1428 M titrant. If these uniform increases do not occur, refer to *Section 3.2.2 Standard Additions* on page *46*.

Summary of Method

After the sample is buffered to pH 10.1, ManVer 2 Hardness Indicator is added, and forms a red complex with a portion of the calcium and magnesium in the sample. EDTA titrant reacts first with the free calcium and magnesium ions, then with those bound to the indicator, causing it to change to a blue color at the end point.

Required Reagents

Description	Unit	Cat. No.
Total Hardness Reagent Set (about 100 tests)		22720-00
Water, deionized	4 L	272-56
Select one or more based on sample concentration		
EDTA Titration Cartridge, 0.0800 M	each	14364-01
EDTA Titration Cartridge, 0.1428 M	each	14960-01
EDTA Titration Cartridge, 0.714 M	each	14959-01
EDTA Titration Cartridge, 0.800 M	each	14399-01

Required Apparatus

Digital Titrator	each	16900-01
Select one or more based on sample concentration		
Cylinder, graduated, 10-mL	each	508-38
Cylinder, graduated, 25-mL	each	508-40
Cylinder, graduated, 50-mL	each	508-41
Cylinder, graduated, 100-mL	each	508-42
Flask, Erlenmeyer, 250-mL	each	505-46

Required Standards

Calcium Chloride Standard Solution, 1000-mg/L as $CaCO_3$	1000 mL	121-53
Calcium Standard Solution, Voluette® Ampule, 10,000-mg/L as $CaCO_3$, 10-mL	16/pkg	2187-10

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Hardness, Calcium

Method 8204

Digital Titrator with EDTA Method

Digital Titrator

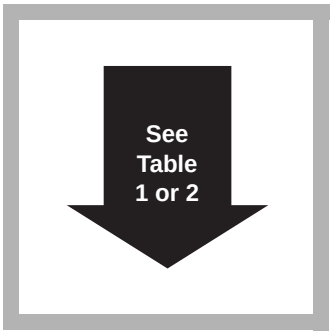
(10 to 4000 mg/L as $CaCO_3$)

Scope and Application: For water, wastewater, and seawater

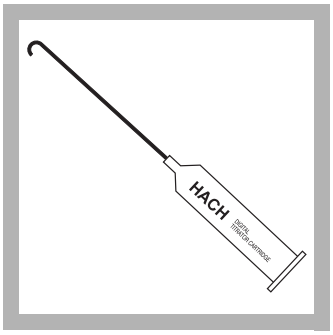
Tips and Techniques

- One German degree of hardness (G.d.h.) = 17.9 mg/L hardness as $CaCO_3$
- You can substitute a 0.1-g scoop of CalVer® 2 Calcium Indicator Powder for the CalVer 2 Calcium Indicator Powder Pillow.
- For added convenience when stirring, use the TitraStir apparatus (Cat. No. 19400-00, -10).
- mg/L Ca = Ca Hardness, mg/L as $CaCO_3$ x 0.40

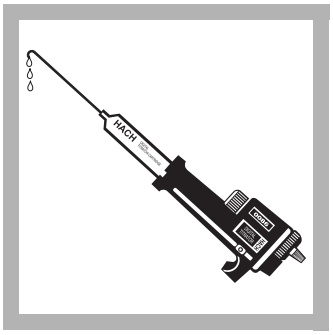
Digital Titration Method 8204



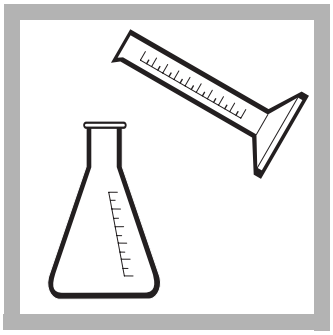
1. Select a sample size and an EDTA Titration Cartridge that corresponds to the expected calcium as calcium carbonate ($CaCO_3$) concentration. Use *Table 1* for concentrations in mg/L and *Table 2* for concentrations in German degrees of hardness.



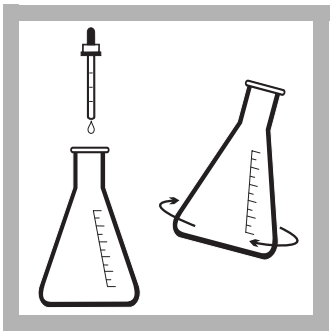
2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator body.



3. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.



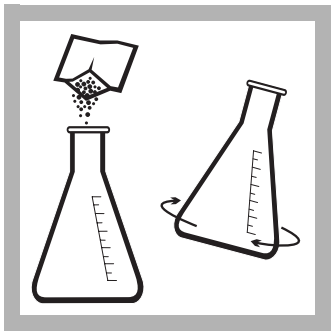
4. Use a graduated cylinder or pipet to measure the sample volume from *Table 1* or *Table 2*. Transfer the sample into a clean 250-mL Erlenmeyer flask. Dilute to about the 100-mL mark with deionized water, if necessary.



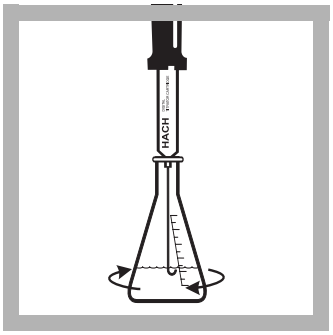
5. Add 2 mL of 8 N Potassium Hydroxide Standard Solution and swirl.

For samples 50 mL and less, add 1 mL.

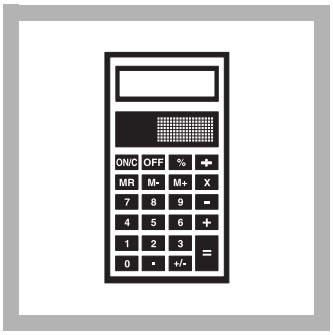
Magnesium is not included in the results but must be present for a sharp end point. If magnesium is absent, add one to two drops of Magnesium Standard Solution, 10-g/L as $CaCO_3$.



6. Add the contents of one CalVer 2 Calcium Indicator Powder Pillow and swirl to mix.



7. Place the delivery tube tip into the solution and swirl the flask while titrating with EDTA from pink to blue. Record the number of digits required.



8. Calculate the sample concentration using one of the formulas below:

Total Digits Required x Digit Multiplier from *Table 1* = mg/L Calcium Hardness as $CaCO_3$

Total Digits Required x Digit Multiplier from *Table 2* = G.d.h.

Table 1

Range (mg/L as $CaCO_3$)	Sample Volume (mL)	Titration Cartridge (M EDTA)	Catalog Number	Digit Multiplier
10–40	100	0.0800	14364-01	0.1
40–160	25	0.0800	14364-01	0.4
100–400	100	0.800	14399-01	1.0
200–800	50	0.800	14399-01	2.0
500–2000	20	0.800	14399-01	5.0
1000–4000	10	0.800	14399-01	10.0

Table 2

Range (G.d.h.)	Sample Volume (mL)	Titration Cartridge (M EDTA)	Catalog Number	Digit Multiplier
1–4	100	0.1428	14960-01	0.01
4–16	25	0.1428	14960-01	0.04
10–40	50	0.714	14959-01	0.1
25–100	20	0.714	14959-01	0.25
>100	10	0.714	14959-01	0.5

Interferences

WARNING: Potassium cyanide is toxic. Always add it after the potassium hydroxide. Excess potassium cyanide does not affect results. Dispose of all cyanide wastes by adding an excess of strongly alkaline sodium hypochlorite solution (bleach) with stirring. Use good ventilation. Allow to stand for 24 hours before disposal.

Some transition and heavy metals complex the indicators and prevent the color change at the end point. Adding a 0.5-g scoop of potassium cyanide (KCN) (Cat. No. 767-14) after adding potassium hydroxide removes interference from the following metals at the levels listed (in an undiluted 100-mL sample), see *Table 3*.

Table 3

Metal	Max. Tolerance Level with KCN	Max. Tolerance Level* without KCN present
Cobalt	20 mg/L	none
Copper	100 mg/L	0.10 mg/L
Nickel	200 mg/L	0.5 mg/L
Zinc	100 mg/L	5 mg/L

* Proportionally higher levels of these elements are tolerable in smaller sample sizes because their effect is diluted when bringing the volume to 100 mL. Because Table 1 and Table 2 have sample volumes of 10–100 mL, the interference concentrations may be greater in your sample and have no effect because of sample dilution

Interfering Substance	Interference Levels and Treatments
Acidity	The test can tolerate 10,000 mg/L acidity.
Alkalinity	The test can tolerate 10,000 mg/L alkalinity and can be run directly in sea water.
Aluminum	Interferes by causing a slow end point but up to 200 mg/L aluminum can be tolerated by allowing sufficient time for the color change.
Barium	Barium is titrated along with calcium but is seldom found in natural waters in significant amounts.
Iron	Interferes above 8 mg/L by causing an orange red to green end point. Accurate results can still be obtained up to about 20 mg/L iron with this end point.
Magnesium	Interference from magnesium is prevented up to 200 mg/L by the formation of magnesium hydroxide at the high test pH but higher levels prevent a distinct end point.
Manganese	Interferes above 5 mg/L.
Orthophosphate	Causes a slow end point but does not interfere if the calcium phosphate that forms is allowed time to redissolve during the titration.
Polyphosphates	Interfere directly and must be absent.
Sodium Chloride	Saturated solutions do not give a distinct end point.
Strontium	Strontium is titrated along with calcium but is seldom found in natural waters in significant amounts.
Temperature	Samples at about 20°C (68°F) or colder should be titrated slowly near the end point to allow sufficient time for the color change.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; see *Section 3.3 Interferences* on page *50*.

Sampling and Storage

If sample cannot be analyzed immediately, add 1.5 mL Nitric Acid (Cat. No. 2540-49) per liter of sample to preserve the sample and to prevent adsorption of the calcium to the container walls. Store in a refrigerator at 4 °C or below; samples preserved in this manner are stable for one week. Neutralize acidified sample to pH 7 with ammonium hydroxide (Cat. No. 14736-32) before testing.

Accuracy Check

Standard Additions Method

Perform this accuracy check when interferences are suspected or to verify analytical technique.

1. Snap the neck off a Hardness Voluette Ampule Standard, 10,000-mg/L as $CaCO_3$.
2. Use a TenSette Pipet to add 0.1 mL of standard to the solution titrated in step 7. Resume titration back to the same end point. Record the number of digits required.
3. Repeat, using two more additions of 0.1 mL. Titrate to the end point after each addition.
4. Each 0.1-mL addition of standard should require 10 additional digits of 0.800 N titrant or 100 digits of 0.0800 N titrant (11 digits of 0.714 M or 56 digits of 0.1428 M titrant).

If these uniform increases do not occur, refer to *3.2.2 Standard Additions*.

Summary of Method

The sample is made alkaline (pH 12–13) with potassium hydroxide to precipitate magnesium as magnesium hydroxide. CalVer 2 Indicator is added and combines with any calcium to form a pink-red color. As EDTA is added, it reacts with the free calcium ions present. When no free calcium ions remain, the EDTA then removes the calcium complexed with the indicator, causing a color change to blue.

Required Reagents

Description	Unit	Cat. No.
Calcium Hardness Reagent Sets (about 100 tests)		
1–16 G.d.h.		24473-00
10–100+ G.d.h.		24474-00
10–160 mg/L		24472-00
100–4,000 mg/L		24475-00
Water, deionized	4 L	272-56

Required Apparatus

Digital Titrator	each	16900-01
Flask, Erlenmeyer, 250-mL	each	505-46
Select one or more based on sample concentration		
Cylinder, graduated, 10-mL	each	508-38
Cylinder, graduated, 25-mL	each	508-40
Cylinder, graduated, 50-mL	each	508-41
Cylinder, graduated, 100-mL	each	508-42

Required Standards

Calcium Standard Solution, Voluette® Ampule, 10,000-mg/L as $CaCO_3$, 10-mL	16/pkg	2187-10

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Appendix B – DR 5000 Apparatus Set, October 2008
Hach PN 2953000

Description	PN	Quantity
2100P Portable Turbidimeter	46500-00	1
7"X 7" hot plate	28816-00	2
AccuVac Snapper	24052-00	2
Acro 50 Vent Filter	26833-18	5
Air Trap Holder Assembly	26639-00	1
Amber Bottle 237 mL	7144-41	1
Ampule breaker	25640-00	1
Beaker Tongs	568-00	2
Beaker, 600 mL	500-52	2
Beaker, poly 150 mL ,12/pk	1080-74	1
Beaker, poly 250 ml, 6/pk	1080-76	1
Beaker, poly 50 mL ,12/pk	1080-71	2
Boat, weighing	21790-00	2
Bottle, wash, poly, 500 mL, 6/pk	26130-49	1
Breaker / Capper Tool for Mercury Scrubber	26640-00	1
Cap for Amber Bottle	21667-06	1
Cell Holder Assembly, TTHM	47880-00	1
C-flex tubing, 0.25 inch ID	23273-67	1
Chlorine & pH Colorimeter	5870012	1
Clamp for Mercury Absorber Column	26562-00	1
Clamp, holder	326-00	4
Clamp, two prong extension	21145-00	2
Clippers, opening PP	936-00	1
Cold Vapor Mercury App Set	26744-00	1
Cotton Balls	2572-01	4
Cylinder, graduated, 10 mL	508-38	2
Cylinder, graduated, mixing, HDPE, 100 mL	2088642	12
Cylinder, graduated, mixing, 25 mL	1896-40	4
Digital Titrator extra delivery tubes	17205-00	30
Digital Titrator for Ca and chloride	16900-01	2
Distilling receiver, 10 ml	26554-38	1
DR 5000 UV-Vis Spec	DR5000SP	1
Dropper	21247-20	2
Dropper 0.5 and 1.0 ml plastic	21247-20	5
Erlenmeyer Flask 250 mL	505-46	2
Evaporation Dish	27647-00	2
Finger Cots, Zetex 2pk	14647-02	2
Flask, Erlenmeyer 500 ml	505-49	2
Flask, Erlenmeyer, 100 ml	26553-42	1
Flask, Erlenmeyer, 2000 mL	24894-54	2
Flask, Erlenmeyer, 250 ml	505-46	2
Funnel, separator, 250 ml	520-46	2
Gas washing bottle, 1200ml	26622-00	1

Glass Elbow, 90-degree with hose adapter	26552-00	1
Graduated cylinder, 100 mL, poly	1081-42	5
Graduated cylinder, 50 mL, poly	1081-41	6
Graduated cylinder, 500 mL, poly	1081-49	5
Graduated cylinder, 25 mL, poly	1081-40	5
Graduated cylinder, 250 mL, poly	1081-46	5
Hach Data Transfer	LZY274	1
Kim Wipes	20970-00	2
LDO probe	LDO0101-05	1
Measuring Spoon, .05 g	49200	2
Measuring Spoon, 0.1g	51100	2
Measuring Spoon, 0.2 g	63800	2
Measuring Spoon, 0.5 g	90700	1
Measuring Spoon, 1.0 g	51000	2
Mercury Absorber Column	26555-10	1
Advanced pH/Conductivity Package	85058-00	2
Microfunnel, poly	25843-35	4
Mixing Cylinder, 50 mL, glass	1896-41	2
Pipette tensette 1.0 - 10.0 ml	19700-10	2
Pipette, tensette 0.1 - 1.0 ml	19700-01	2
Pipette, volumetric 5 ml	14515-37	2
pipette,wiretrol, 50 -100 uL	25689-05	1
Precision Balance - Zeta Series 500g Capacity	29371-01	2
Ring Stand	563-00	4
Ring, support	580-00	1
Sample Cell 1" round, 10 mL, w/cap	24276-06	4
Sample Cell 1" square, 10 & 25 mL	26659-08	1
Sample Cell, 1" 10 & 25 mL w/caps	26126-02	5
Sample Cell: 5 cm Rect Glass, 50 mm Pathlength	26292-50	1
Sample cells 10-20-25 w/ cap	24019-06	5
Sample Cells, 1" square, 10 mL matched pair	24954-08	1
Separator Funnel	1406149	2
Spoon, measuring	907-00	1
spoon, measuring 0.05 g	492-00	1
spoon, measuring 0.2 g	638-00	1
StablCal Cal. Stds.	26594-05	1
Stir Bar Retriever	15232-00	2
Stir Bar, Magnetic	20953-50	2
Stir Bar, Magnetic	20953-55	2
Stopper for 18 mm Tube, 25/pk	1731-25	4
Stopper for distilling receiver	26559-00	1
Stopper for Gas Washing bottle	26623-00	1
Stopper, hollow	14480-00	3
Support Base and rod	329-00	1
support ring	580-01	2
Support ring for Gas Washing bottle	26563-00	1
Support, Separator Funnel	22386-00	2
Tensette Pipette Tips 1.0 - 10 ml	21997-25	4
Tensette Pipette Tips 0.1 - 1.0 ml	21856-28	1
Test tube Rack	18641-00	1
Thermometer	26357-02	2

Tilting Dispenser	22200-38	1
Titrastir Stir Plate	19400-00	2
Tubing Quick Disconnect, HDPE	14810-00	1
Vacuum Pump	28248-00	1
Volumetric Flask 100 mL, poly	14060-42	6
Volumetric Flask 200 mL, polypropylene	14060-45	6
Volumetric Flask 500 mL, polypropylene	14060-49	6
Volumetric flask, polypropylene,1000 mL,	14060-53	2
Watch Glass, Pyrex	578-67	1
Water bath and rack	1955-55	4

Appendix C - DR 5000 Reagent Set, October 2008
Hach PN 2953100

Reagent Set	Description	PN	Quantity
Arsenic	Arsenic Test Kit	28000-00	1
	Arsenic Std Soln	14571-42	1
	Hydrochloric Acid, concentrated	134-49	1
Barium	BariVer 4 Powder Pillows	1206499	1
	Barium Std Soln, 5000 mg/l	14611-42	1
Cadmium	Dithiver Metals Rgt PP pk/100	1261699	1
	Buffer Pwd Plw, Citrate pk/100	1420299	1
	Chloroform, ACS 4L	1445817	1
	Sodium Hydroxide Solution 50% 500ml	218049	4
	Cotton Balls, Absorbent pk/100	257201	1
	Potassium Cyanide, 125g	76714	1
	Cadmium Std Solution 100mg/L	1402442	1
Chromium, Total	Total Chromium Reagent set	22425-00	1
	Chromium Trivalent Std 50mg/L	1415142	1
Copper	CuVer AccuVac	25040-25	4
	Copper Std Solution 100mg/L	128-42	1
Cyanide	Cyanide Reagent Set	24302-00	1
	Potassium Cyanide ACS grade 125 g for Std.	767-14	1
Fluoride	Fluoride AccuVac	25270-25	4
	Fluoride Std Soln, 1 mg/l	291-49	1
Hardness, Ca Titration	Calcium hardness Rgt set 10 - 160	24472-00	1
	Calcium hardness Rgt set 100 - 4000	24475-00	1
	Hardness Quality Control Std, low range, 500mL	2833449	1
	Hardness Quality Control Std, high range, 500mL	2833349	1
Hardness, Total Titration	Total hardness Rgt Set	22720-00	1
	EDTA Titration Cartridge .08 M	14364-01	1
	EDTA Titration Cartridge .8 M	14399-01	1
Lead	LeadTrak Rgt Set	23750-00	5
	10 mg/L Lead Standard Solution	23748-20	10
Mercury	Cold Vapor Mercury Rgt Set	26583-00	4
	Hydroxylamine Hydrochloride	246-14	2
	Nitric Acid	152-49	4
	Potassium Permanganate	168-01H	2

	Potassium Persulfate	26175-01	2
	Sulfuric Acid	979-09	2
	Mercury Std Solution 1000mg/L	14195-42	1
Nitrate	Nitrate Nitrogen Std soln 2ml ampoule	14260-20	2
	TNT nitraVerX RGT set	26053-45	2
	Nitrate Nitrogen Std Soln, 10-mg, 500mL	307-49	1
	Nitrate Nitrogen Std soln, Volutte, 500-mg	14260-10	1
Nitrite	TNT NitriVer 3 Nitrite RGT Set	26083-45	2
	Sodium Nitrite	2452-01	2
Selenium	Toluene, ACS 4L	1447017	1
	Titraver Hardness Reagent, ACS 100g	20426	1
	Potassium Hydroxide 12N 100ml	23032	1
	Buffer Solution, Sulfate type 500ml	45249	1
	Diaminobenzidine 4-Hydrochloride	706222	1
	Selenium Std soln	22407-42	1
Total Trihalomethanes	TTHM reagent set	27908-00	1
	Chloroform Ampoules	27567-07	12
	Organic Free Water	26415-49	2
Chloride	Silver Nitrate Method Chloride Rgt set	22880-00	2
	Chloride std soln voluette	14250-10	6
Sulfate	SulfaVer rgt set AccuVac	25090-25	4
	Sulfate std solution 50 mg/l amps	2578-49	6
Color (Apparent)	Hydrochloric Acid Soln. 1.0 N	23213-53	1
	Sodium Hydroxide, 1.0 N	1045-53	1
	Color Standard 15N	26028-53	1
Alachlor	Immunoassay reagent set	28130-00	5
Atrazine	Immunoassay reagent set	27627-00	5
TPH	Immunoassay reagent set, TPH in soil	28143-00	2
Other Chemicals:	Hydrochloric Acid, concentrated	134-49	2
	Sodium Hydroxide, 5 N	2450-53	2
	Sulfuric Acid,1.00N	1270-32	2
	Water de-ionized	27256	8

Appendix D – Water Laboratory Setup Checklist

- ☐ Complete Inventory of reagents and glassware against SOP Listings – Record discrepancies.
- ☐ Check expiration date on all reagents - record expired reagents and/or reagents that will expire within 60 days (submit requisition for replacement upon completion of laboratory setup).
- ☐ Clean, acid rinse and air dry all glassware/plasticware according to procedures below.
- ☐ Power up DR 5000 and allow instrument to perform self-check.
- ☐ Run & record Reagent Blanks
- ☐ Run five (5) randomly selected standard solutions - if result of testing is within +/- 20% of the concentration of the standard, the lab is ready to receive samples.
- ☐ Setup water sample log. See Appendix E for an example of a sample water log.
- ☐ Report that the Water Laboratory is operational and/or any limitations in operational status to OIC.

Procedure of Acid Washing Glassware/Plasticware

- Rinse glassware with tap water.
- Clean glassware with a solution of water and laboratory detergent.
- Don chemical resistant gloves and face shield
- Rinse the glassware with a 1:1 solution of hydrochloric acid (HCl)
- Rinse glassware at least 3X with de-ionized water.

Note – take care not to scratch or etch sample cells while cleaning!

Making a 1:1 HCl Solution

A 1:1 HCl solution contains 15%-25% HCl by weight. To approximate a 1:1 HCl solution, mix equal parts of concentrated HCl (Hach part # 134-49) and de-ionized water.

- Don chemical resistant gloves and face shield
- Measure equal parts de-ionized water and concentrated HCl
- Place de-ionized water into a container large enough to rinse glassware/plasticware
- Slowly add HCl to the water to minimize splashing

Appendix E – Sample Water Log

Date/time of sample collection	Collecting unit	Date Received	Unique Sample ID #	Base camp name	Sample location (DFAC, head, etc.)	pH & chlorine residual at time of collection		Sample preserved? (√)		Date of analysis	QA/QC complete i.e., Standard addition (√)		Analysts' name(s)
						pH	Cl^-	Yes	No		Yes	No	